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Journal of the Fisheries Research Board of Canada

Announcement

An author–subject index and list of all publications issued during 1974 will be supplied in early 1975, and distributed free to all Journal recipients, including subscribers. Additional copies will be available through the Office of the Editor.

Avis

Un index par sujet et auteur et une liste de toutes les publications parues en 1974 paraîtront au début de 1975 et seront distribués gratuitement à tous ceux qui reçoivent le Journal, ainsi qu'aux abonnés. On pourra se procurer des exemplaires supplémentaires au Bureau du directeur de la publication.

J. C. STEVENSON
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Identification, Analysis, and Removal of Geosmin from Muddy-Flavored Trout

M. YURKOWSKI AND JO-ANNE L. TABACHEK

Department of the Environment, Fisheries and Marine Service
Freshwater Institute, Winnipeg, Man. R3T 2N6

YURKOWSKI, M., AND J. L. TABACHEK. 1974. Identification, analysis, and removal of geosmin from muddy-flavored trout. J. Fish. Res. Board Can. 31: 1851–1858.

Geosmin has been isolated and identified, using gas-chromatographic, mass-spectrometric, and organoleptic analysis, as the principal compound responsible for the muddy flavor in fish from various saline lakes in western Canada. A method for quantitative estimation of geosmin has been described and evaluated. Placing of muddy-flavored rainbow trout (*Salmo gairdneri*) in clean water resulted in reduction of flavor to the borderline level between 3 and 5 days, and of geosmin concentration from 1.1 to 0.3 μg/100 g of flesh in 14 days. The taste threshold level of geosmin was estimated to be 0.6 μg/100 g flesh.

YURKOWSKI, M., AND J. L. TABACHEK. 1974. Identification, analysis, and removal of geosmin from muddy-flavored trout. J. Fish. Res. Board Can. 31: 1851–1858.

Les auteurs ont isolé et identifié, par chromatographie en phase gazeuse, spectrométrie de masse et analyse organoleptique, la géosmine comme étant le principal composé responsable du goût de vase de poissons provenant de divers lacs salins de l'ouest du Canada. Ils décrivent et évaluent une méthode d'estimation quantitative de la géosmine. Le fait de placer les truites arc-en-ciel (*Salmo gairdneri*) dans de l'eau claire a pour effet de réduire le goût aux bornes de la catégorie entre 3 et 5 jours et d'abaisser la teneur en géosmine de 1.1 à 0.3 μg/100 g de chair en 14 jours. Le niveau de seuil de goût est estimé à 0.6 μg de géosmine/ 100 g de chair.

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SINCE the report on the muddy or earthy odor in soil by Berthelot and André (1891), the problem of muddy odor or flavor in surface waters (Rushton 1929; Adams 1929; Burger and Thomas 1934; Issatchenko and Egorova 1944; Ferramola 1949; Silvey et al. 1950; Silvey and Roach 1953, 1956; Morris 1962; Lewis 1966; Kikuchi and Mimura 1972) and in fish (Cornelius and Bandt 1933; Thaysen 1936; Balon 1950; Aschner et al. 1967; Lovell 1972) has been reported from various parts of the world. The source of this muddy flavor can be attributed to the growth of some actinomycetes, especially *Streptomyces* (Rullmann 1895; Adams 1929; Thaysen 1936; Issatchenko and Egorova 1944; Ferramola 1949; Ilnyckyj 1951; West 1953; Romano and Safferman 1963; Hansen 1964; Silvey and Roach 1956; Dickson 1968), and species of blue-green algae (Cornelius and Bandt 1933; Aschner et al. 1967; Lewis 1966; Silvey and Roach 1959; Kekwick 1970, quoted in Rosen et al. 1970) in water. The cause of this odor is apparently due to the production of geosmin by either actinomycetes or blue-green algae or both, and methylisoborneol by actinomycetes (Gerber and Lechevalier 1965; Gerber 1967, 1968, 1969; Dougherty et al. 1966; Safferman et al. 1967; Medsker et al. 1968, 1969; Rosen et al. 1968, 1970; Henley et al. 1969). These two compounds have been isolated and identified from surface water (Rosen et al. 1970).

Studies have been done on the muddy-tasting fish in Saskatchewan (Balon 1950; Ilnyckyj 1951; West 1953) subsequent to complaints from commercial fisherman who had harvested tainted fish that were not marketable. The source of the taste was attributed to metabolites from *Streptomyces* occurring in freshwater and saline lakes. Although a crude fraction containing an odorous compound(s) was isolated, its identity was not established (Ilnyckyj 1951). In our studies in Manitoba and that of the Department of Natural Resources in Saskatchewan (M. Atton personal communication) on the utilization of saline lakes for the production of different species of fish, we have encountered muddy flavor in fish from some of the lakes. Since muddy flavor can be a problem in fish farming of

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saline lakes, it was therefore necessary to identify, and to modify a method of assay to be applicable for, the compound(s) caussing muddy flavor in fish. In addition, a preliminary experiment on the removal of this flavor from live fish, using the assay method described and organoleptic analysis, was done. Studies on the source of the odorous compounds in saline waters will be the subject of another paper.

Materials and Methods

Isolation of Muddy Odor Compound from a Soil Organism

A soil-isolated organism, *Streptomyces* sp., was grown in Czapek-Dox medium (2.0 g $NaNO_3$, 0.5 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 1.0 g KH_2PO_4 and 50.0 g glucose/liter of water) using 12-liter Florence flasks under aseptic aeration (glass column-packed with mineral oil-impregnated glass wool) for 7 days. The filtered growth medium (9 liters) was distilled until 500 ml of distillate was collected. The distillate was extracted twice with 25 ml of methylene chloride. The extract was reduced to 2 ml under nitrogen. The major odor compound was isolated by preparative gas chromatography as described below.

Isolation of a Muddy Odor Compound from Fish

Fish, 50–500 g in size, were obtained from various saline lakes in Manitoba and Saskatchewan for qualitative analysis of muddy flavor. Immediately after arrival, the fish were minced and a composite sample of 250 g was placed in a 5-liter distilling flask containing 2.5 liters of boiled distilled water. The odor present in the fish was distilled over and extracted from the distillate (250 ml) twice with 10 ml of methylene chloride. The extract was reduced to 2 ml under nitrogen. The compound responsible for the muddy flavor was isolated by preparative gas chromatography from the extracts of the most intensely flavored fish samples.

Gas Chromatography

The muddy odor peaks from the soil-isolated organism and fish samples were identified by smelling the effluent from a gas chromatograph (Varian Aerograph, model 2100), equipped with a glass column (182 cm × 6 mm OD, 1.5 mm ID) packed with 3% OV-1 on Gas-Chrom Q. The injection inlet had to be fitted with a teflon-lined septum. For preparative work and smelling of effluent peak an effluent splitter (1:10) was used. The column was run at ambient temperatures (30 C) for 5 min and then temperature programmed from 30 to 200 C at 6 degrees/min. The injector and detector temperatures were set at 220 and 240 C, respectively. The flow rates of helium, hydrogen, and air were set at 25, 25, and 250 ml/min, respectively. The attenuation × range was set at 8×10^{-12} amp/mv. The muddy odor peaks were collected in a glass capillary according to the technique of Burson and Kenner (1969), where air, passed through liquid nitrogen, was used to cool the capillary. The collected peaks were run again to remove any impurities still present. The isolated odor compounds were analysed by a Finnigan mass spectrometer.

Quantitative Estimation of Geosmin in Fish

For the estimation of geosmin concentration in fish, a high-vacuum low-temperature distillation apparatus (Keay and McGill 1968) was modified as shown in Fig. 1. It was necessary to close off the distillate receiver C to prevent the loss of odor during thawing and extraction.

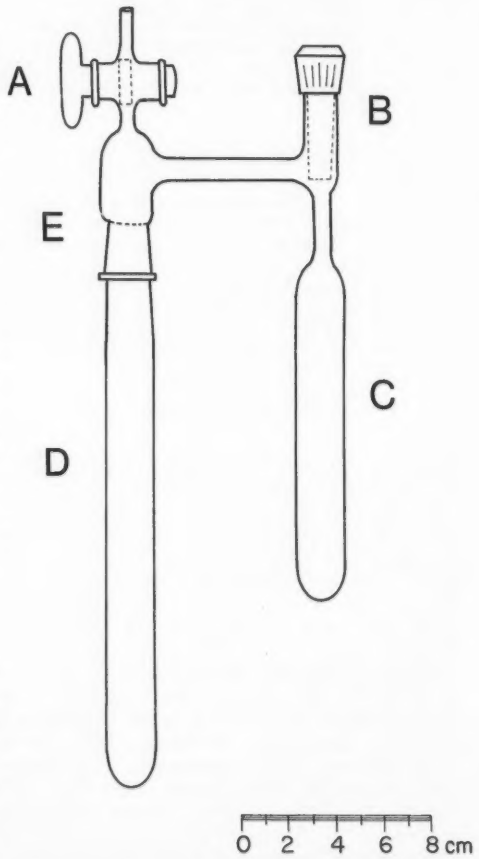

Fig. 1. High-vacuum low-temperature glass distillation apparatus, scaled down to half size. A = stopcock; B = Rotoflo stopcock, TFG/18; D = test tube containing fish sample; C = distillate receiver; E = Clearfit joints (KSRB 19[female] and KCNB 19[male] made by Quickfit). Stopcock A is connected to vacuum pump.

The modification of the Keay and McGill procedure for the isolation and assay of geosmin in fish is described as follows: A 12-g sample of frozen fish was cut into thin pieces about 2 mm thick and placed in test tube D. To the test tube joint high vacuum grease (volatiles removed under high vacuum at 250 C) was applied and the tube attached to the female joint E of the apparatus. When stopcocks A and B were closed, tube D, containing the sample, was placed in liquid nitrogen for ½ h. Then the vacuum from the two-stage vacuum pump-air pump (Welch Duo-Seal, model 1405), which produces a vacuum up to 0.05 μ (5×10^{-5} mm Hg), was applied to the outlet of stopcock A. It is advisable to have a trap cooled with liquid nitrogen between the stopcock and the vacuum pump. This stopcock was then opened and the apparatus evacuated for 30 s. Then the Rotoflo stopcock B was opened and the evacuation was continued for 2.5 min. Stopcock A was closed and liquid nitrogen was taken away from tube D and used for the partial immersion of distillate receiver C. The sample in tube D was immersed in water at 65–75 C and kept at this temperature until most of the water in the sample was distilled over. The temperature was then increased to 95 C and allowed to remain at this temperature for 3 h. Stopcock B was closed, both the water and liquid nitrogen baths were removed, and receiver C was immersed in water at 65–70 C to partially melt the ice inside in order to prevent cracking of the receiver. When the contents of the receiver reached room temperature, stopcock B was removed and 200 μl of carbon disulfide containing a known amount of methyl heptadecanoate internal standard (16 μg/ml) was added. Stopcock B was closed, the contents were shaken well, and the carbon disulfide was allowed to settle at the bottom of the tube. The carbon disulfide extract, along with some aqueous layer to cover the top of the carbon disulfide layer, was transferred to 0.5-dr vials with teflon-lined screw caps. A desired volume of carbon disulfide layer was used for the gas chromatographic estimation of geosmin.

The concentration of geosmin in fish (C), expressed in micrograms/100 g tissue, was calculated by the formula $C = (100 \times P \times S)/(A \times W)$ where P = peak area of unknown, S = concentration of internal standard added (micrograms), A = peak area of standard, and W = weight of sample (grams). The relative response ratios of geosmin to the internal standard (methyl heptadecanoate) were not determined due to quantitites of pure geosmin available being too small to allow accurate weighing. All results expressed are to be considered relative to the internal standard.

Organoleptic Analysis

The fish samples obtained from various lakes were analyzed organoleptically in the following manner. Each fish was cut into two or more steaks which were wrapped in aluminum foil and a hole punched in the foil. The packages were placed on a stand above the water level inside a pan with boiling water for 15 min. Immediately after cooking, a panel of two to six people

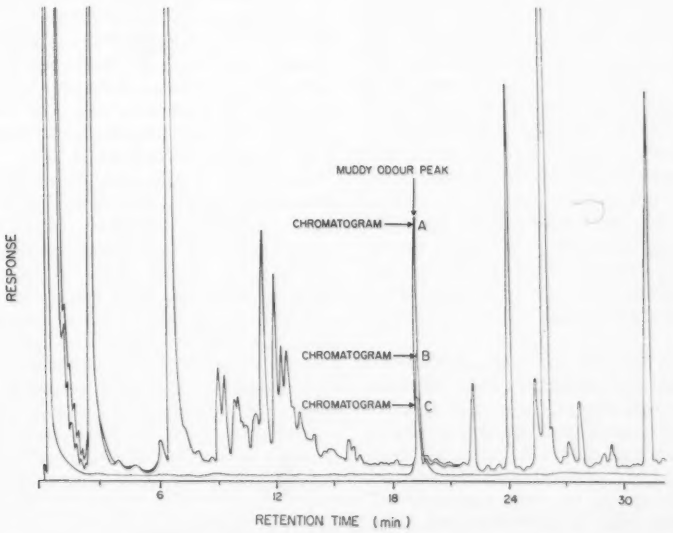

FIG. 2. Superimposed gas chromatograms showing muddy odor peaks from the (B) compound isolated from *Streptomyces* distillate, (A) rainbow trout (*Salmo gairdneri*) extract spiked with B, and (C) rainbow trout extract, same volume as used in A.

with unknown taste threshold levels each received up to six packages of steak from different fish and determined the presence or absence of muddy flavor. At least a day prior to the analysis the panelists were given a solution containing the muddy odor compound isolated from *Streptomyces* sp. to smell in order that they could identify the muddy flavor.

Removal of Geosmin from Live Rainbow Trout

Rainbow trout grown in Smuts Lake, Alvena, Saskatchewan, were placed in cages in the Ketchum reservoir near Saskatoon, Saskatchewan, for one month in order that the fish would develop a strong muddy flavor. They were brought to this Institute and placed in a flow-through tank. The chlorinated water from the City of Winnipeg was treated with sodium thiosulfate prior to use. Samples of six fish were taken at 0, 1, 2, 3, 5, 7, and 14 days and were analyzed for geosmin both quantitatively and organoleptically. Six samples from each fish were analyzed organoleptically by six different people. The fish were classified by the panel as strong, slight, borderline, or clear.

Results and Discussion

Identification of Geosmin in Fish

We initially noted that the odor and flavor of so-called muddy fish obtained from various lakes appeared to be similar to the odor emanating from the *Streptomyces* sp. isolated from the soil. When the odor extract from the soil microorganism and the fish were separated on gas-chromatograph equipment with a splitter, and effluent peak from the fish (peak C) had the same retention time and odor as that from the soil microorganism (peak A) (Fig. 2). Furthermore, when the fish extract was spiked with a compound collected from the muddy odor peak from *Streptomyces* extract, the muddy odor peak of the fish (peak C) increased in height (see peak B). It was found later that the authentic sample of geosmin, obtained from Dr Gerber, had the same characteristic odor and retention time as that from the compound isolated from the *Streptomyces* sp.

To confirm that the isolated odor components from the fish and soil organisms were identical compounds, they were analyzed by mass spectrometry. These two isolated components produced identical mass spectra as shown in Fig. 3. Furthermore, the mass spectra of these two components were essentially identical to that published for natural geosmin (Medsker et al. 1968; Rosen et al. 1970; Kikuchi and Mimura 1972) and *dl*-geosmin (Kikuchi and Mimura 1972) synthesized according to the method of Marshall and Hochstetler (1966). There appears to be no doubt that geosmin, probably

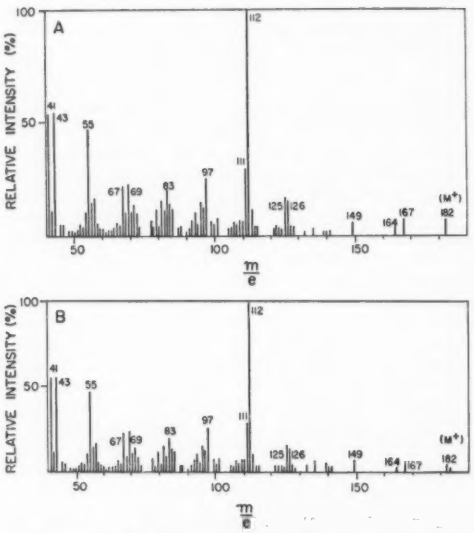

Fig. 3. Mass spectra of (A) unknown muddy odor compound isolated from fish tissue and (B) muddy odor compound from soil-isolated microorganisms, *Streptomyces* sp.

of a structure of *trans*-1,10-dimethyl-*trans*-9-decalol as established by Gerber (1968), is responsible for the muddy flavor in fish from saline lakes in western Canada. This does not rule out that other compounds with similar or even different odor characteristics can occur and taint the fish. For example, a compound(s) like methylisoborneol could taint the fish if it is present in these waters.

According to organoleptic analysis (Table 1), it was found that some saline lakes in Saskatchewan and Manitoba contained muddy-flavored fish, while others did not. From smelling of gas-chromatographic effluent peaks, it was found that all odor extracts from both muddy-flavored and non-muddy-flavored fish contained geosmin. This suggests that all untainted fish grown in western Canada could contain geosmin at levels below the taste threshold level. The results in Table 1 also show that depending on the lake, the fish may be tainted throughout, or for a short period of time during any portion of the growing season which usually lasts from early May to late October.

Analysis of Geosmin

The method developed for the analysis of geosmin in fish was tested for reproducibility and recovery of geosmin in fish (Table 2). Three different volumes

of geosmin solution (an unknown amount dissolved in hexane) in quadruplicate were added directly to the internal standard solution (200 µl CS_2 containing known amount of methyl heptadecanoate), which was covered with boiled distilled water. The same volumes of geosmin solution were added to the

TABLE 1. Detection of geosmin, using gas-chromatographic effluent odor technique and organoleptic analysis, in rainbow trout (*Salmo gairdneri*) taken from some of the saline lakes in Manitoba and Saskatchewan.

Date	Name of body of water	Geosmin	
		G.C. effluent odor technique[a]	Organoleptic analysis[b]
July 23/69	Nesland L. (Mt.Nebo, Sask.)[c]	+	+
July 21/69	Lake 100[d]	+	-
Sept. 4/69	" "	+	+
Oct. 29/69	" "	+	-
Oct. 1969	Whitebear Reservoir (Sask.)	+	-
Oct. 1969	Cleland Reservoir (Sask.)	+	-
Oct. 1969	Ketchum Reservoir (Sask.)	+	+
June 1970	" "	+	+
July 1970	" "	+	+
Aug. 1970	" "	+	+
Sept. 1970	" "	+	+
June 1970	Smuts L.(Alvena, Sask.)	+	-
July 1970	" "	+	-
Aug. 1970	" "	+	-
Sept. 1970	" "	+	-
Aug. 1970	Transfer from Smuts L. to Ketchum Reservoir	+	+
June 1970	Lake 154		-
July 1970	Lake 154		-
Oct. 1970	Lake 154		-
July 22/71	Lake 888		+
July 22/71	Lake 940		+
June 10/71	Lake 958		+
Sept. 7/71	Lake 104		+
Aug. 1972	Lake 958		+
Aug. 1972	Lake 318		+
Aug. 1972	Lake 926		+
Oct. 1972	Lake 586		-
Oct. 23/72	Lake 624		+
Aug. 1971	Lake 786		+
Sept. 1972	Lake 513		+
Sept. 1973	Lake 255		+
Sept. 1973	Lake 876		+
Sept. 1973	Lake 624		+
Sept. 1973	Lake 108		-
Sept. 1973	Lake 111		-
Sept. 1973	Lake 300		-
Sept. 1973	Lake 303		-
Sept. 1973	Lake 312		-
Sept. 1973	Lake 321		-

[a]Odor detected had same retention time as geosmin.

[b]Positive sign (+) shows that geosmin was detected and negative sign (–) that it was not detected.

[c]This lake contained kokanee.

[d]Numbering system of lakes in the Erickson, Manitoba, area that are being used by the Freshwater Institute in the experimental fish farming program. Maps of this area may be obtained from this Institute.

TABLE 2. Reproducibility and recovery of geosmin added directly to the internal standard and to fish.

Volume geosmin added μl (concn not known)	μg geosmin estimated: Added directly to internal standard	μg geosmin estimated: Added directly to fish in apparatus	% recovery
2.0	0.59	0.38	64
2.0	0.59	0.39	66
2.0	0.70	0.26	37
2.0	0.58	0.25	45
Mean±SE	0.62±0.028	0.32±0.038	53±7.1
3.7	1.37	0.63	46
3.7	1.40	0.51	36
3.7	1.43	0.58	41
3.7	1.34	0.53	36
Mean±SE	1.44±0.037	0.57±0.025	40±2.4
4.7	1.62	1.28	80
4.7	1.64	0.81	49
4.7	1.69	0.65	38
4.7	1.63	0.83	31
Mean±SE	1.65±0.016	0.89±0.118	55±8.3

fish sample, containing an undetectable amount of geosmin, that was placed in tube D of the glass distillation apparatus (Fig. 1). The geosmin recovered from fish and that added directly to the internal standard were analyzed by gas chromatography. Results show that excellent reproducibility was obtained among the quadruplicates when geosmin was added to internal standard solution. However, when the same levels of geosmin were added to the fish samples variable results were obtained; the recovery of geosmin was 40–55% on the average with a range from 36 to 80%. However, the reproducibility of results from three different muddy-flavored rainbow trout, assayed in duplicate, was much better (Table 3).

TABLE 3. Reproducibility in analysis of muddy-flavored fish in duplicate.

Sample no.	μg geosmin/100 g fish
1	0.66
1	0.59
Mean±SE	0.63±0.035
2	0.48
2	0.46
Mean±SE	0.47±0.010
3	0.90
3	0.85
Mean±SE	0.87±0.015

There are a number of factors which could affect the recovery of geosmin and the reproducibility of results obtained by this method. The recovery of geosmin in the carbon disulfide extract depends on the temperature of the distillation and condensation portions of the apparatus, which affects the amount of geosmin transferred from sample to collection tube, the loss during evacuation and addition of internal standard due to the ease of sublimation (high vapor pressure) of geosmin, and efficiency of the one extraction used. It is essential that conditions of assay be standardized in order to obtain reproducible results.

Although six analyses per day can be performed by one person using six distillation units with this complex method, a simpler, faster, and more accurate method would be desirable, especially one that could be used in the field. This would supplement on a quantitative basis the present organoleptic method used in the detection of muddy flavor in fish, which is dependent on the taster's threshold level for geosmin.

REMOVAL OF GEOSMIN FROM LIVE FISH

The level of geosmin in rainbow trout when placed in clean water decreased with time (Table 4).

TABLE 4. Analysis of geosmin and muddy flavor level in tainted rainbow trout placed in continuous flow tank using City of Winnipeg water supply.

Time in clean water, days	Geosmin concn μg/100 g fish	Range in geosmin levels	Flavor
0	1.1±0.11(6)[a]	0.8–1.5	21/36[b](strong)
1	1.0±0.06(6)	0.7–1.2	21/36 (strong)
2	0.8±0.06(6)	0.6–1.0	6/36 (strong)
3	0.8±0.04(6)	0.7–0.9	9/36 (slight)
5	0.5±0.06(6)	0.3–0.7	3/36 (borderline)
7	0.4±0.03(6)	0.3–0.5	8/36 (borderline)
14	0.3±0.02(6)	0.2–0.3	1/36 (borderline)

[a]Mean ±SE. Number in brackets shows number of fish sampled.
[b]This means that muddy odor was detected in 21 out of 36 (6 fish × 6 replicates) samples.

The concentration of geosmin decreased on the average from 1.1 to 0.3 μg/100 g of fish flesh in 14 days. On each sampling period, there was a variability of results attributable to the method of analysis and individual variations in the fish.

According to organoleptic analysis (Table 4) the muddy flavor of the fish became very faint or borderline on about the fifth day after treatment and remained at about the same level thereafter. This borderline level of muddy odor may be attributed to the water supply, which could have had a faint smell and taste of geosmin during the entire experiment, but was unnoticed prior to the seventh day of treatment. It is therefore likely that the muddy flavor from rainbow trout could be removed in 5–7 days. This is dependent on the water supply and the concentration of the odor compound present in the fish. This is in agreement with the studies on rainbow trout (Thaysen and Pentelow 1936) and channel catfish (Lovell and Sackey 1973) where it was shown that by placing fish in contaminated water under laboratory conditions and subsequently into clean water, the muddy flavor from the trout was removed in 5 days and that of the catfish in 15 days. These results are probably dependent primarily on the initial levels of muddy odor compounds present in the fish and on fish species. The work of Thaysen and Pentelow (1936) strongly suggests that the muddy flavor enters the fish principally through the gills and is transported to the muscle by the blood stream. Their work rules out the skin as an absorption site but not that of the digestive tract. The results of Lovell and Sackey (1973) suggest that the digestive tract is also an important absorption site as indicated by a more intense taint of catfish which had ingested the muddy compound-synthesizing organisms.

From both quantitative and organoleptic analysis it appears that the taste threshold level of geosmin in the rainbow trout was about 0.3 μg/100 g of flesh or 3 ppb. On the assumption that 50% geosmin is recovered, the threshold level would be about 6 ppb. In water the taste threshold level of geosmin was reported to be 0.2 ppb (Safferman et al. 1967; Rosen et al. 1968) and 0.05 ppb (Medsker et al. 1968).

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Theory of Size Distribution in Ecological Communities[1]

S. R. KERR

Ontario Ministry of Natural Resources
Fish and Wildlife Research Branch, Maple, Ont.

KERR, S. R. 1974. Theory of size distribution in ecological communities. J. Fish. Res. Board Can. 31: 1859–1862.

Observed regularities in the size composition of pelagic ecosystems are explained by a theoretical model based on trophic processes. The model assumes, in accord with available evidence, that prey and predator sizes are simply related, and that growth and metabolism satisfy similar functions of body (particle) size when averaged over entire trophic levels. The model appears suited to other community types that satisfy these conditions, and suggests that trophic processes are complementary to competitive displacement in determining that particle density is a linear function of the logarithm of particle size.

KERR, S. R. 1974. Theory of size distribution in ecological communities. J. Fish. Res. Board Can. 31: 1859–1862.

Nous expliquons la régularité observée dans la distribution des tailles dans des écosystèmes pélagiques par un modèle fondé sur des processus trophiques. Le modèle suppose, en accord avec l'évidence disponible, que la taille des proies est en relation simple de celle des prédateurs, et que la croissance et le métabolisme satisfont les mêmes fonctions de grosseur corporelle (des particules), lorsqu'on fait la moyenne de niveaux trophiques entiers. Le modèle semble applicable à d'autres types de communautés qui remplissent ces conditions, et donne à croire que les processus trophiques sont complémentaires du déplacement compétiteur, en montrant que la densité des particules est fonction linéaire du logarithme de la grosseur des particules.

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RELATIVELY little attention has been directed to the size compositions of ecological communities taken as integral units. Clearly, it would be of great interest to know whether overall community size composition is constrained within definite patterns.

Size composition is evidently predictable within single trophic elements of certain terrestrial ecosystems (e.g. Brown 1973; Brown and Lieberman 1973), but evidence spanning a number of trophic levels is not abundant, apart from the extensive observations of marine pelagic communities reported by Sheldon et al. (1972). The latter report is remarkable for the important regularities observed over a wide range of particle diameters (1–10^6 μm). Particle concentrations (in ppm, by volume) were found to be relatively uniform when the data were grouped in logarithmic size intervals, with some indication of a slight (approximately twofold) decrease in concentration with increasing logarithmic intervals of particle size.

It is not immediately obvious why whales, for example, should manifest approximately the standing concentrations of phytoplankton, although Sheldon et al. (1972) offered a partial explanation. The model developed in the present paper shows that a general explanation of their observations lies in the direction they suggested, an explanation that appears relevant to communities other than those of the marine pelagic zone.

The Model

In this section I develop a model of the size distribution of organisms in a generalized food chain. A useful representation of the energy budget for an individual animal has been provided by Winberg (1956), in the form

$$pR = T + \Delta W$$

where R represents the total ration corrected by p for nonassimilation, T is metabolism, and ΔW rep-

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resents growth in terms of the portion of assimilated rations retained as body substance, all determined over common intervals of time. The equivalent equation for a population is

$$pNR = N(T + \Delta W) \quad (1)$$

where N is the number of individuals comprising the population, p is redefined to account for unutilized together with nonassimilated prey production, and the remaining variables are population averages of the variables defined above. If equation 1 represents the energy budget for a prey population, it follows that $N\Delta W$ defines the average ration that is potentially available to its predators. Pursuing this reasoning, the production of trophic level i provides potential forage for trophic level $i+1$ at a rate described by

$$N_i\Delta W_i = N_{i+1}R_{i+1} \quad (2)$$

where the variables are defined as above except that each now represents an average calculated over all populations comprising a given trophic level.

Substituting equation 2 into 1, and subscripting as appropriate, gives the energy budget for trophic level $i+1$ as

$$p_{i+1}N_i\Delta W_i = N_{i+1}(T_{i+1}+\Delta W_{i+1}) \quad (3)$$

where the coefficient p corrects for all prey production that is lost, directly or indirectly, to decomposers. Equation 3 rearranges to the form

$$\frac{N_i}{N_{i+1}} = \frac{T_{i+1}+\Delta W_{i+1}}{p_{i+1}\Delta W_i} \quad (4)$$

I now derive an alternate formulation for the growth rate, ΔW. Figure 13 provided by Sheldon et al. (1972), and fig. 9 of Sheldon et al. (1973), indicate that the time required for marine organisms to double their body sizes is adequately described by a power function. That is,

$$\text{doubling time} = aW^b$$

where W is body weight and a and b are fitted coefficients. A general measure of growth rate is provided by the ratio of average body size to doubling time, and then

$$\Delta W = kW^{1-b} \quad (5)$$

where $k = 1/a$.

Recalling that metabolism is adequately described by the familiar power function of body weight,

$$\bar{\imath} = \alpha W^{\gamma}$$

where α and γ are fitted coefficients, equation 4 can now be rewritten as

$$\frac{N_i}{N_{i+1}} = \frac{\alpha_{i+1}W_{i+1}^{\gamma}+kW_{i+1}^{1-b}}{p_{i+1}kW_i^{1-b}} \quad (6)$$

On average, it is not unlikely that prey and predator sizes are related in some relatively simple way. The simplest supposition (discussed below) is that prey size, on average, constitutes a consistent fraction of predator size. If so, then

$$W_i = qW_{i+1}$$

where q represents the postulated fraction of proportionality, and then it follows that equation 6 can be restated in terms of W_{i+1}. That is,

$$\frac{N_i}{N_{i+1}} = \frac{\alpha_{i+1}W_{i+1}^{\gamma}+kW_{i+1}^{1-b}}{p_{i+1}kq^{1-b}W_{i+1}^{1-b}}$$

which rearranges to the form

$$\frac{N_i}{N_{i+1}} = \frac{\alpha_{i+1}W_{i+1}^{\gamma+b-1}}{p_{i+1}kq^{1-b}} + \frac{1}{p_{i+1}q^{1-b}}$$

Multiplying through numerator and denominator by the identities W_i and qW_{i+1}, respectively, and removing q from the LHS, gives

$$\frac{N_iW_i}{N_{i+1}W_{i+1}} = \frac{q^b}{p_{i+1}}\left[\frac{\alpha_{i+1}W_{i+1}^{\gamma+b-1}}{k} + 1\right] \quad (7)$$

For present purposes, equation 7 provides an adequate statement of the model.

Evaluation of the Model

Equation 7 indicates that the biomasses of any two adjacent trophic levels will be in constant proportion if the power index of body size approximates zero, i.e. if $\gamma \simeq 1-b$. This condition asserts that the rates of growth and metabolism of various organisms respond similarly, on average, to changes in body size. As with other functional responses of organisms, it is entirely reasonable that growth rate should reflect the metabolic rate, and then equivalent functional responses of growth and metabolism to organism size would be expected. The empirical evidence supports this view.

The metabolism index, γ, has been estimated by various workers for a wide variety of organisms; the great majority of estimates are in the order of 0.7–0.9, with 0.8 being a representative value for

aquatic poikilotherms (see, Winberg 1956; Kleiber 1961; Paloheimo and Dickie 1966). From fig. 13 of Sheldon et al. (1972) and fig. 9 of Shedlon et al. (1973), I estimate, in terms of body weigth, that the power index $b = 0.2$ (assuming that particle diameter varies as the cube root of particle weight). It follows that $\gamma+b-1$ approximates zero, and according to equation 7, the biomasses of adjacent trophic levels therefore exist in simple proportion to each other, essentially independent of body size.

It remains as a matter of some interest to estimate the constant of biomass proportionality between adjacent trophic levels. Suitable generalized data are lacking, but an approximation is achievable. The power indices γ and b have been given above in reaching the conclusion that body size does not appreciably affect calculations based on equation 7. The coefficient q can be estimated on the basis that a typical prey organism might average 5% of the body length of its predator, suggesting that $q = .000125$ in terms of body weight. The coefficient p has been estimated to equal 0.8 for some groups of organisms (Winberg 1956), but without making allowance for the additional nonutilization losses directly to decomposers that would occur in nature. Accordingly, I have used 0.7 as a more conservative estimate of p.

The available data are insufficient for establishing a reliable measure of central tendency for the ratio $\alpha:k$. An approximation that is appropriate to entire trophic systems can be derived by noting that the energy equivalent of growth is likely to be in the order of 20% of ingested energy, for many organisms. If so, $\alpha:k$ is in the order of 4. Substituting the foregoing estimates in equation 7 indicates that the standing stock of a trophic level should typically be in the order of 1.2 times that of its predators, a conclusion that is closely consistent with the empirical data provided by Sheldon et al. (1972).

Discussion

The foregoing evaluation indicates that equation 7 provides an adequate representation of the processes that determine size distribution in marine pelagic communities. The model should apply equally to other ecological communities that satisfy the necessary conditions.

In its present formulation, the model is clearly adequate to the extent that its various coefficients are indeed relatively constant. The extensive literature in comparative physiology provides assurance that the coefficients that are essentially physiological do in fact seem to vary relatively little among various taxonomic groups.

The coefficient q, relating the relative sizes of predator and prey, is not directly physiological, and could vary so extensively in some environments as to render an average value meaningless. A constant value of q implies a uniform trophic structure, in which predation typically occurs as whole-body ingestion of prey organisms. An obvious exception occurs in terrestrial ecosystems, where primary consumers such as insects would clearly not exhibit q values comparable to their predators. In general, it seems likely that q would not be reliably constant in communities that utilize solid substrate, because of the associated opportunity for greatly varied morphological structure, hence variable q, particularly among primary producers.

Analysis of the predation process indicates that relative prey size is an important determinant of predator size and efficiency (Schoener 1969; Kerr 1971a, b), and of the community size structure that results (Kerr 1974). Conversely, predation has been shown to affect the size composition of prey resources (Galbraith 1967; Brooks and Dodson 1965). Taken together, both factors suggest that observed size relations between elements of a food chain are a consequence of size-dependent feedback between predator and prey stocks. Trophic level is associated in general with the sizes of aquatic organisms (Kerr and Vass 1973), thus it follows that relative sizes of predator and prey in pelagic communities can be represented, as a meaningful average, by the single coefficient q. I infer that equation 7 is appropriate in its present form only to pelagic ecosystems, although provision of empirically derived functions describing the relation of prey and predator size could readily be incorporated in the model, broadening its applicability.

The present model is based on idealized trophic relationships. Natural food chains are typically less clear than their idealized representations, owing to changing trophic roles as organisms grow. Hutchinson (1959) has provided the term "metaphoetesis" as a convenient descriptor of the blurred food chains we typically observe in nature. In some cases the data provided by Sheldon et al. (1972) indicate clear density modes at approximately tenfold intervals of particle diameter, suggesting relatively distinct trophic levels, but the majority of their data indicate that metaphoetesis obscures clear trophic distinctions. The model assumes that trophic levels can be assigned as discrete integer values to the organisms in a natural community. A trophic level would be more appropriately assigned to an organism as a decimal number derived by taking a weighted mean of the trophic levels of its various prey species. A continuous model could then be derived, rather than the discrete version provided as equation 7, but this was not attempted here for reasons of ease and clarity of presentation.

The present model is "coarse-grained," in the sense that predation is considered, but not the competitive processes that also affect size composition within relatively narrow size strata of a community. The evidence for size-displacement between species that utilize similar food resources is relatively abundant, and has been reviewed recently (MacArthur 1972). It is sufficient to note that competitive size-displacements of approximately twofold, in terms of body weight, seem typical. Assuming that food abundance is relatively uniform within a specific resource category, it follows that competitive interactions result in logarithmic (multiplicative) size distributions of competing predators. That is, the overall semilogarithmic distribution reflected in equation 7 can be thought of as comprising a large number of comparable distributions occurring within specific trophic elements.

The present model points to three conclusions that seem worth considering. First, the model itself, together with the possible elaborations discussed above, promises to provide a general framework for the identification and analysis of size-dependent regularities in natural communities. The existence of predictable constraints on the permissible patterns of size composition in ecological communities suggests a potential for deriving practical criteria for community stability and integrity. Second, the model suggests that community size composition may be determined by processes that are essentially species-independent, in the sense that ecological roles seem more influential than typological distinctions in the present context. Finally, the model suggests a practical formulation for assessing the effects of exogenous stress on ecological communities. Woodwell (1970), Regier and Loftus (1972), and Kerfoot (1974) have noted the tendency to reduced average size in stressed communities. The present model provides a quantitative means to evaluate the effects of such stresses.

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Redescription of *Anonchohaptor anomalum* Mueller 1938 and a Description of *Icelanonchohaptor fyviei* n.sp. (Monogenoidea: Dactylogyridae)[1]

ALEX O. DECHTIAR

Fish and Wildlife Research Branch
Ontario Ministry of Natural Resources, Maple, Ont.

AND WILLIAM A. DILLON

Department of Biological Sciences
The University of Tennessee at Martin, Martin, Tenn. USA

DECHTIAR, A. O., AND W. A. DILLON. 1974. Redescription of *Anonchohaptor anomalum* Mueller 1938 and a description of *Icelanonchohaptor fyviei* n.sp. (Monogenoidea: Dactylogyridae). J. Fish. Res. Board Can. 31: 1863–1866.

Icelanonchohaptor fyviei n. sp., from the gills of quillback, *Carpiodes cyprinus* (LeSueur), is described and *Anonchohaptor anomalum* Mueller 1938 is redescribed. A new host record, longnose sucker, *Catostomus catostomus* (Forster), is reported for *Anonchohaptor anomalum* Mueller 1938.

DECHTIAR, A. O., AND W. A. DILLON. 1974. Redescription of *Anonchohaptor anomalum* Mueller 1938 and a description of *Icelanonchohaptor fyviei* n.sp. (Monogenoidea: Dactylogyridae). J. Fish. Res. Board Can. 31: 1863–1866.

Nous décrivons *Icelanonchohaptor fyviei* n.sp., provenant des branchies de la couette, *Carpiodes cyprinus* (LeSueur), et redécrivons *Anonchohaptor anomalum* Mueller 1938. Nous signalons un hôte nouveau, le meunier rouge, *Catostomus catostomus* (Forster), d'*Anonchohaptor anomalum* Mueller 1938.

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THREE species of *Anonchohaptor* from North American fishes have been described: *A. anomalum* by Mueller (1938), *A. muelleri* by Kritsky et al. (1972), and *A. olseni* by Leiby et al. (1973).

Mueller (1938) erected the genus *Anonchohaptor* to include *A. anomalum* and assigned the genus to the family Calceostomatidae (Parona and Perugia 1890) Poche 1926. Bychovsky (1957) and Yamaguti (1963) reviewed the taxonomic status of *Anonchohaptor* and concluded that this genus should be retained in the family Calceostomatidae. Gussev (1969) removed the genus *Anonchohaptor* (as well as *Acolpenteron* Fischthal and Allison 1941) from Calceostomatidae and included it in the family Dactylogyridae. Kritsky et al. (1972) and Leiby et al. (1973) also included *Anonchohaptor* in Dactylogyridae.

Leiby et al. (1972) erected the genus *Icelanonchohaptor*, a genus closely related to *Anonchohaptor* and placed it in the family Dactylogyridae. The genus *Icelanonchohaptor*, prior to this report, included *I. icelanonchohaptor* Leiby, Kritsky, and Peterson 1972, from the gills of *Ictiobus cyprinellus* (Val.), bigmouth buffalo and *I. microcotyle* Kritsky, Leiby, and Shelton 1972, from the gills of *Carpiodes carpio* Rafinesque, river carpsucker.

We concur at this time that *Anonchohaptor* and *Icelanonchohaptor* belong in the family Dactylogyridae. The subfamilial status, however, is questionable. The subfamilial status of these two genera will be the subject of a separate paper.

Materials and Methods

During 1968–71, 60 specimens of four species of catostomid fishes were collected: 15 quillback and 15 white sucker, *Catostomus commersoni* (Lacépède), (Lake Erie); 15 shorthead redhorse, *Moxostoma macrolepidotum* (LeSueur), (Lake St. Clair); 15 longnose sucker (*C. catostomus*) (Lake Huron). Hosts were captured with hoop or gillnets by staff of our research

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stations. Nasal apertures, branchial material, and the body of each fish were placed separately into a 1:4000 formalin solution according to the method of Putz and Hoffman (1963). After 1 h, the parasites were removed from the sediment for immediate examination or were placed in a 10% formalin solution for future study. The worms were stained with Gomori's trichome and mounted in glycerine jelly. Measurements are given in millimeters; the mean is presented first, followed by the minimum and maximum in parentheses. Illustrations were made with the aid of a camera lucida and photomicrography.

Results and Discussion

Anonchohaptor anomalum Mueller 1938 (Fig. 1-12)

HOSTS AND LOCALITIES: *Catostomus commersoni* (Lacépède) white sucker from Lake Erie; *C. catostomus* (Forster) from Lake Huron

HABITAT: Gills

PREVIOUSLY REPORTED HOSTS AND LOCALITIES: *Catostomus commersoni* (Lacépède), northern hog sucker, *Hypentelium nigricans* (LeSueur), and black redhorse from Chautauqua Lake, New York (Mueller 1938), *Moxostoma duquesnei* (LeSueur); *Hypentelium nigricans* (LeSueur) from Rock Creek, Fannin County, Georgia (Chien 1969); *Catostomus commersoni* (Lacépède), *Carpiodes cyprinus* (LeSueur), silver redhorse, *Moxostoma anisurum* (Rafinesque), and *M. macrolepidotum* (LeSueur) from Lake Erie (Dechtiar 1972); *Carpiodes cyprinus* (LeSueur), *Catostomus commersoni* (Lacépède), and *Moxostoma anisurum* (Rafinesque) from Lake of the Woods (Dechtiar 1972a).

NUMBER STUDIED: 10

REDESCRIPTION: Body slightly tapered posteriorly; tegument smooth (Fig. 1). Body 3.119 (1.886–4.000) long by 0.611 (0.377–1.811) wide. Cephalic lobes divided; cephalic lappets prominent. Head organs present (Fig. 1, 9); 8–10 large cephalic glands in each bilateral group, extending from midpharynx to copulatory complex. Mouth subterminal. Pharynx subspherical; transverse diameter 0.341 (0.246–0.556), longitudinal diameter 0.347 (0.279–0.485). Esophagus elongate, bifurcating immediately anterior to copulatory complex; intestine U-shaped (Fig. 2, 10). Four eyespots present; posterior pair farther apart. Peduncle short. Posthaptor disc-like, well separated from body; transverse diameter 0.410 (0.284–0.509). Posthaptor with 14 hooks, two central and 12 arranged peripherally; hooks similar in size and morphology; hooks 0.017 (0.015–0.021) long; accessory sclerotized process as in Fig. 3. Gonads situated near body midlength. Testis spherical, postovarian; vas deferens loops left intestinal crus; distal end of vas deferens expanded to form seminal vesicle. Two prostatic reservoirs entering proximal end of cirrus, one about 3 times as long as the other. Ovary sac-like, passing around right intestinal crus; oviduct short, fusing with the seminal receptacle duct and vitelline duct prior to entering ootype. Ootype surrounded by large Mahlis' glands; uterus a large, thin-walled tube. Vaginal complex (Fig. 4) dextroventral; vaginal pore surrounded by a cuticularized rim; thin, sinuous tube passing medially to join anterior portion of seminal receptacle. Copulatory complex consisting of a basally articulating cirrus and accessory piece. Cirrus (Fig. 5), a U-shaped tapering tube with prominent bend at the distal end; an angular projection present about midway of the cirrus; cirrus 0.048 (0.039–0.056) long. Accessory piece (Fig. 6) with two arms; 0.054 (0.045–0.063) long. Vitellaria consisting of irregularly shaped follicles arranged in two massive lateral bands; absent in region of gonads and copulatory complex. Transverse vitelloducts fusing medially. Eggs pyriform (Fig. 7, 8), 0.251 (0.153–0.345) long by 0.163 (0.125–0.222) wide.

DISCUSSION: The above redescription is given because the original description is incomplete. The redescription should aid in future comparative studies.

During the second part of May, 1970 we collected from the gills of the white sucker (Lake Erie) three pairs of *A. anomalum* in the state of copulation (Fig. 10–11). Copulation of monogeneans is known only for a small number of species (Bychovsky 1957). When sexually mature pairs copulate, they may be facing in the same direction or in opposite directions.

Icelanonchohaptor fyviei n.sp. (Fig. 13-21)

HOST: *Carpiodes cyprinus* (LeSueur), quillback

HABITAT: Gills

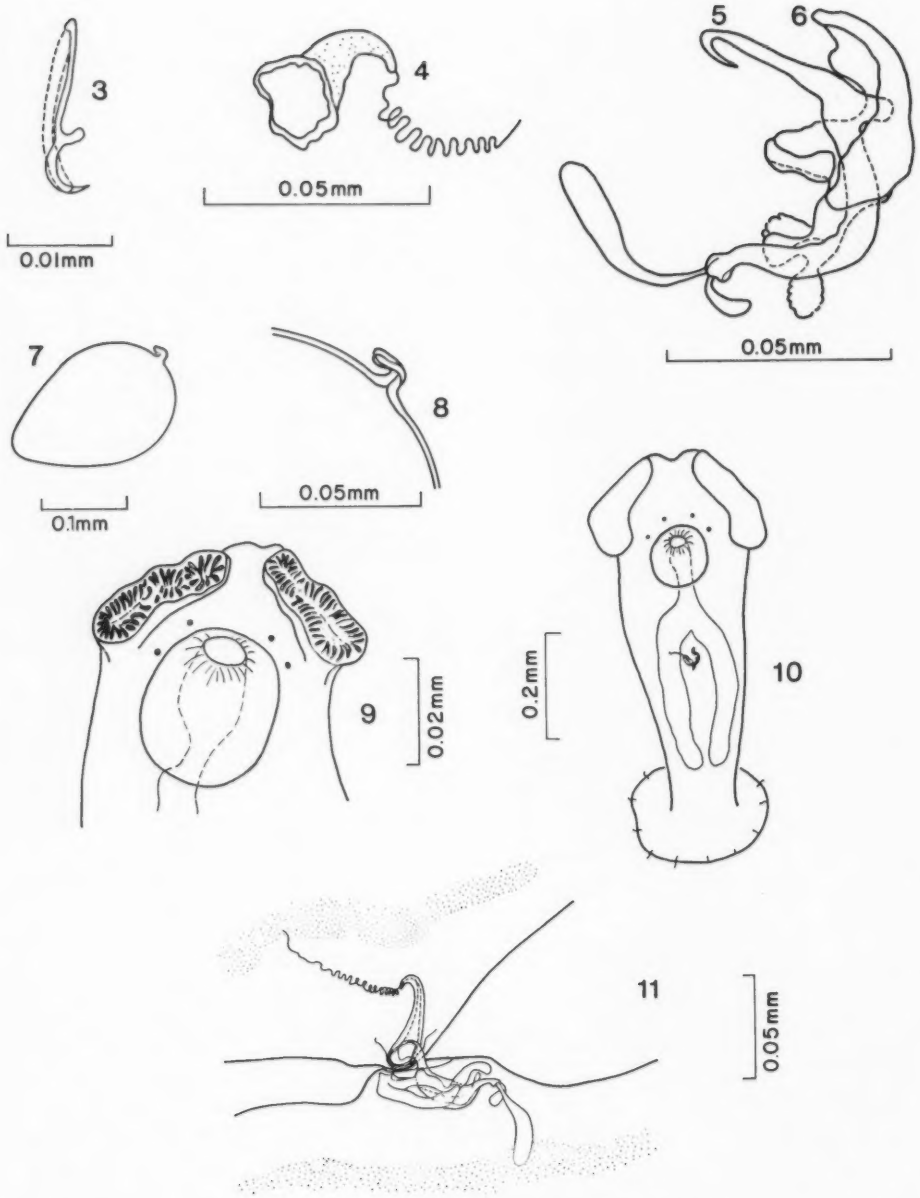
TYPE LOCALITY: Lake Erie

NUMBER STUDIED: 10

TYPE SPECIMENS: Holotype, U.S.N.M. Helm. Coll. No. 73199. Paratypes (2), U.S.N.M. Helm. Coll. No. 73200; remaining paratypes in senior author's collection.

FIGURES 1 and 2.

FIG. 1. *Anonchohaptor anomalum*, dorsal view of monogenean (×90); FIG. 2. *Anonchohaptor anomalum*, dorsal view of monogenean (young) showing U-shaped intestine (×170).



FIGURES 3–11. *Anonchohaptor anomalum* Mueller 1938

FIG. 3. Hook; FIG. 4. Vagina; FIG. 5. Cirrus; FIG. 6. Accessory piece; FIG. 7. Egg with a knob; FIG. 8. Knob of the egg; FIG. 9. Anterior end of monogeneans with head organs; FIG. 10. Young monogeneans with U-shaped intestine; FIG. 11. Copulation between two monogeneans.

FIG. 12. *Anonchohaptor anomalum* in the state of copulation (×110).

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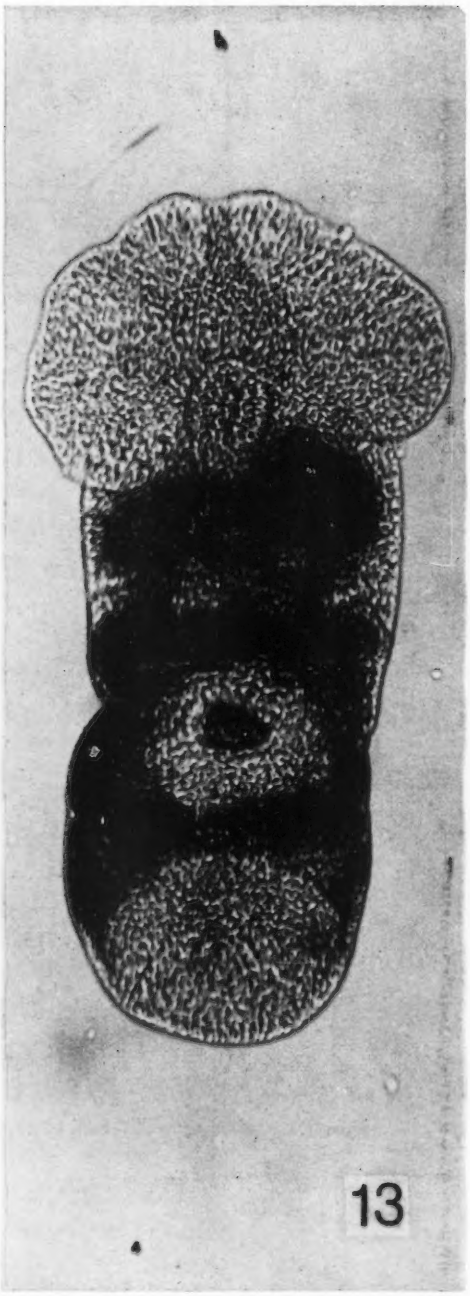
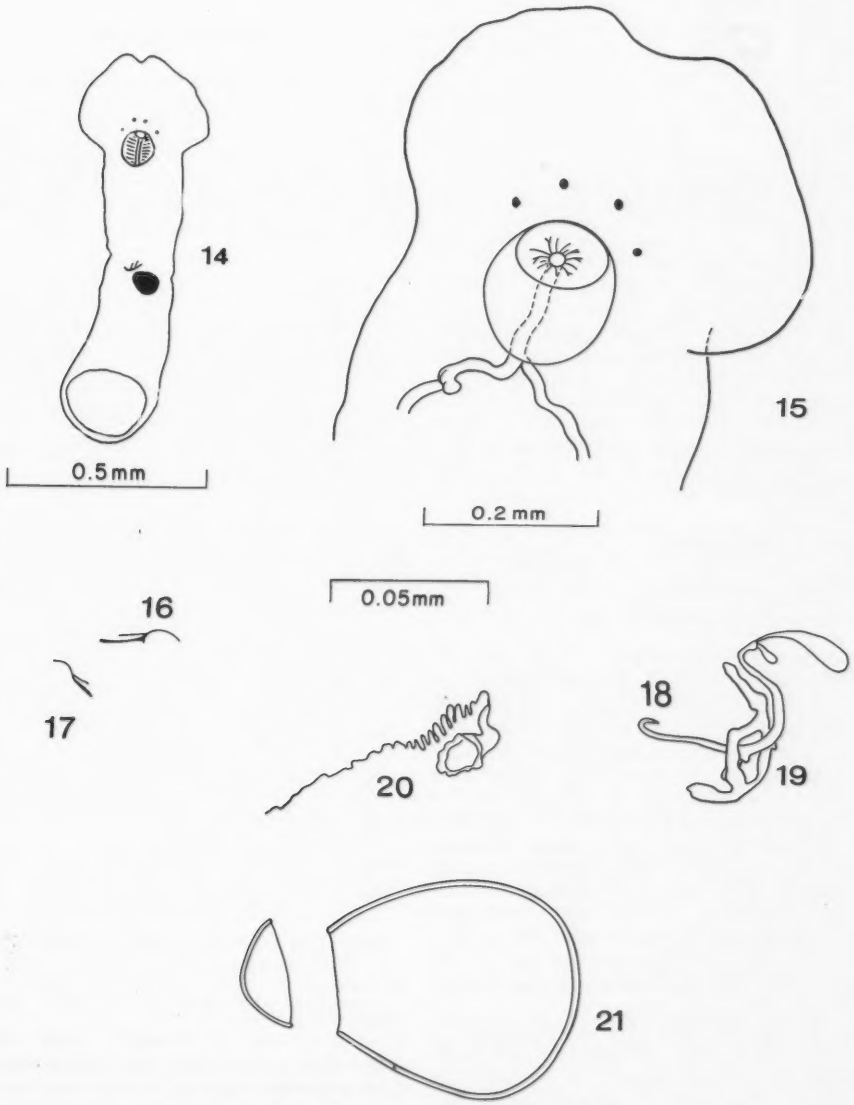


FIG. 13. *Icelanonchohaptor fyviei* n.sp. dorsal view of monogenean (×110).

DESCRIPTION: Body tapered posteriorly to the midlength (Fig. 13–14); body 0.993 (0.768–1.198) long by 0.264 (0.189–0.368) wide (on anterior end of trunk). Tegument thin and smooth. Cephalic lobes poorly developed with numerous head organs and two pairs of cephalic pits. Bilateral groups of 8–10 large cephalic glands present. Four eyespots present; posterior pair farther apart. Mouth (Fig.



FIGURES 14–21. *Icelanonchohaptor fyviei* n.sp.
FIG. 14. *Icelanonchohaptor fyviei* n.sp. (dorsal view); FIG. 15. Anterior end of monogenean; FIG. 16. Hook (central); FIG. 17. Hook (marginal); FIG. 18. Cirrus; FIG. 19. Accessory piece; FIG. 20. Vagina; FIG. 21. Egg with operculum.

15) subterminal; pharynx round or slightly oval, 0.117 (0.080–0.156) in diameter; esophagus elongate, bifurcating immediately posterior to pharynx; gut U-shaped. Peduncle short and wide. Posthaptor cap-like, 0.171 (0.103–0.249) in diameter. Posthaptor with 14 hooks; 12 distributed around the margin and 2 central; hooks similar in shape, dissimilar in size (Fig. 16, 17); central hooks 0.024 (0.022–0.025) long; marginal hooks 0.015 (0.014–0.017) long. Copulatory complex composed of cirrus and accessory piece. Cirrus (Fig. 18) a curved, hollow, U-shaped tube with an attenuated sharp point and an enlarged base; angular projection present about midway of cirrus; two prostatic reservoirs empty into proximal end; cirrus 0.032 (0.026–0.040) long. Accessory piece (Fig. 19) with two arms; 0.032 (0.027–0.038) long. Ovary elongate, looping right intestinal crus; greatest width 0.091 (0.083–0.099). Vagina on right margin of trunk with heavily sclerotized opening (Fig. 19); cuticularized vaginal tube sinuous; seminal vesicle ovoid. Testis ovoid, postovarian; vas deferens loops left intestinal crus; diameter of testis 0.063 (0.055–0.068). Vitellaria coextensive with intestine except in region of gonads; absent in region of gonads. Eggs pyriform, operculate, 0.081 (0.076–0.086) long by 0.052 (0.047–0.078) wide (Fig. 21).

NAME: *Icelanonchohaptor fyviei* n.sp. is named in memory of the senior author's colleague Dr A. Fyvie, former parasitologist for the Research Branch, Department of Lands and Forests, Maple, Ont.

DISCUSSION: *Icelanonchohaptor fyviei* n.sp. can be distinguished from *I. icelanonchohaptor* as follows: (1) body 0.768–1.198 long by 0.189–0.368 wide rather than 3.93–5.78 long by 0.792–1.060 wide; (2) pharynx 0.080–0.156 in diameter rather than 0.341–0.561; (3) posthaptor smaller; (4) posthaptor hooks dissimilar in size rather than similar (central hooks 0.022–0.025 long and peripheral hooks 0.014–0.017 rather than all being 0.018–0.019); (5) testis 0.055–0.068 in diameter rather than 0.187–0.242; (6) copulatory complex differs in morphology; (7) cirrus 0.026–0.040 long rather than 0.056–0.087; (8) accessory piece 0.027–0.038 rather than 0.064–0.098; (9) number of cephalic glands; (10) eggs 0.076–0.086 long rather than 0.112–0.135; and (11) host.

The new species differs from *I. microcotyle* as follows: (1) body 0.768–1.198 long by 0.189–0.368 wide rather than 2.7–4.6 long by 0.401–1.008 wide; (2) pharynx 0.080–0.156 in diameter rather than 0.231–0.440; (3) central posthaptor hooks 0.022–0.025 long rather than 0.016–0.019; (4) testis 0.055–0.068 in diameter rather than 0.148–0.231; (5) copulatory complex differs in morphology; (6) cirrus 0.026–0.040 long rather than 0.045–0.062; (7) accessory piece 0.027–0.038 long rather than 0.064–0.084; (8) ovary 0.055–0.068 in diameter rather than 0.099–0.209; and, (9) host.

Acknowledgments

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Behavior of Juvenile Coho Salmon (*Oncorhynchus kisutch*) Exposed to Sumithion (Fenitrothion), an Organophosphate Insecticide

CHRISTOPHER J. BULL

Department of Recreation and Conservation
Fish and Wildlife Branch, Victoria, B.C.

AND JOHN E. MCINERNEY

Department of Biology, University of Victoria, Victoria, B.C.

BULL, C. J., AND J. E. MCINERNEY. 1974. Behavior of juvenile coho salmon (*Oncorhynchus kisutch*) exposed to Sumithion (fenitrothion), an organophosphate insecticide. J. Fish. Res. Board Can. 31: 1867–1872.

Wild underyearling coho salmon, *Oncorhynchus kisutch*, in a stream aquarium were exposed to sublethal concentrations of the organophosphate insecticide Sumithion (0.10, 0.23, 0.48, and 0.75 ppm fenitrothion). After 2-h exposure at 0.48 and 0.75 ppm the pattern of behavior changes indicated physiological impairment. All behaviors involving locomotion, including some comfort behaviors, virtually ceased. Many fish, unable to maintain position, were swept downstream. Only coughing increased in frequency with insecticide concentration, providing an indicator of physiological stress. In the 0–0.23 ppm range social behaviors and feeding markedly decreased in frequency whereas comfort movements generally increased; feeding was depressed at concentrations as low as 0.1 ppm. Of the various behaviors examined, feeding probably provides the best combination of a sensitive indicator of low level contamination by an organophosphate insecticide and a behavior with important ecological implications.

BULL, C. J., AND J. E. MCINERNEY. 1974. Behavior of juvenile coho salmon (*Oncorhynchus kisutch*) exposed to Sumithion (fenitrothion), an organophosphate insecticide. J. Fish. Res. Board Can. 31: 1867–1872.

Nous avons exposé des saumons coho (*Oncorhynchus kisutch*) sauvages de moins d'un an à des concentrations sublétales de l'insecticide organophosphoré Sumithion (0.10, 0.23, 0.48 et 0.75 ppm de fenitrothion) dans un aquarium à eau courante. Après une exposition de 2 h à 0.48 et 0.75 ppm, les changements de comportement indiquent un affaiblissement physiologique. Tous les comportements impliquant la locomotion, y compris certains comportements de confort, cessent pratiquement. Plusieurs poissons, incapables de maintenir leur position, sont entraînés par le courant. Seule la toux devient plus fréquente à mesure qu'augmente la concentration de l'insecticide, fournissant un indicateur utile du stress physiologique. Dans l'intervalle de 0–0.23 ppm, les comportements sociaux et alimentaires diminuent notablement de fréquence, alors que les mouvements associés au confort deviennent en général plus fréquents: l'alimentation diminue à des concentrations aussi basses que 0.1 ppm. Parmi les divers comportements étudiés, l'alimentation est probablement l'indicateur le plus sensible d'un faible niveau de contamination par un insecticide organophosphaté, et est en même temps un comportement qui a d'importantes implications écologiques.

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SPRAGUE (1971) has noted that behavioral changes provide sensitive indicators of pollutants in sublethal concentrations and has stressed the need to evaluate the ecological significance of such changes. The present work examines a wide range of behavior changes in underyearling coho salmon exposed to sublethal concentrations of Sumithion (fenitrothion: *0,0*-dimethyl *0*-(4-nitro-*m*-tolyl) phosphorothioate), an organophosphate insecticide widely used in forest spraying (Freitag et al. 1969).

Young Atlantic salmon (*Salmo salar*) exposed by Symons (1973) to 1 ppm fenitrothion for 15–16 h and then placed in uncontaminated water in a

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stream aquarium showed a 50% decline in territory holding, an effect which persisted for 2–3 wk. The aim of the present work was to identify specific behaviors which are sensitive indicators of low concentrations of the insecticide and have important ecological implications. Observations were made with wild fish in a stream aquarium which simulated most of the physical conditions which young coho would face if subjected to forest spraying.

Materials and Methods

For each test 16 underyearling coho were seined from the Goldstream River (near Victoria, B.C.) between 0930 and 1030 and transferred within 1 h to a stream aquarium with similar environmental conditions. The fish rapidly resumed apparently normal activities: in control tests between 1300 and 1700 the same day, agonistic behaviors reached frequencies similar to those recorded by Mason and Chapman (1965) and Hartman (1965a).

The aquarium (Hartman 1965b) was divided by screens (10 meshes/inch) into four similarly furnished observation sections (2.5 m long × 0.6 wide × 0.7 deep). Four fish of different sizes (range 37–79 mm and 0.4–5.5 g) were placed in each section giving a density of 2.7 fish/m², a figure close to natural coho densities observed by Chapman (1962) of 3.0 fish/m². Individual fish were identified by position and size. Physical conditions closely simulated those of the Goldstream collection site. The water, from a drainage system which includes the Goldstream River, was slightly alkaline (pH 7.3) and low in dissolved solids (29 ppm). Washed gravel (1–2.5 cm diameter) and approximately 10 rocks (10–30 cm) covered the bottom of each section. Current velocities ranged from 0 to 25.5 cm/s and temperature was controlled at 11±1 C. Floodlights provided subsurface intensities of 45–428 lux between 0830 and 1730 and daylight before and

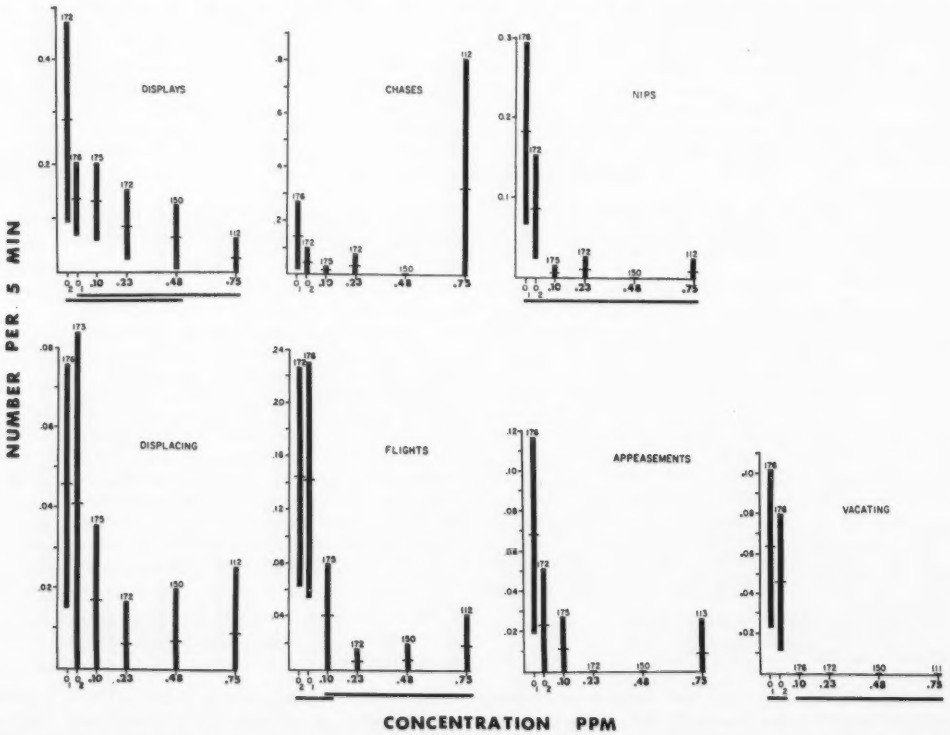

FIG. 1. Frequencies of various agonistic behaviors of underyearling coho salmon (*Oncorhynchus kisutch*) at several concentrations of fenitrothion (means for four tests, 22 h observation and four fish each, and 95% confidence limits). The insecticide concentrations are corrected values; see text for details. Numerals above bars are numbers of scores on which means are based. Responses not underscored by the same line under the X-axis differ at $P < 0.05$ (Sign test). In tests where no overall significance was found (Friedman's two-way anova) no lines appear under the X-axis.

after these times provided seasonal photoperiod changes. Dissolved oxygen was consistently near 100% saturation. A constant rate feeding apparatus daily distributed 10 g of freshly thawed brine shrimp, *Artemia salina*, into the aquarium over a 24 h period.

Sumithion (53% fenitrothion in an emulsifier consisting of 37–42% xylene and 5–10% Sorpol (a mixture of polyoxyethylene alkylaryl ester, polyoxyethylene sorbitan alkylate, and an anionic sulfate)) was chosen because it is a widely used spray preparation. The biological effects observed in this study may have been due in part to the emusifier or to synergistic effects. Five concentrations were tested corresponding to 0, 7.3, 18.0, 36.5, and 57.8% of the 96 h median lethal concentration of the insecticide (1.30 ppm) as established by three standard bioassays (Bull 1971). Each concentration was tested once and the control twice in the sequence 0_1, 0.23, 0.48, 0.75, 0.10, and 0_2 ppm during the period Oct. 26–Nov. 28, 1970. The insecticide was added to the aquarium 15 h before the fish.

Gas-chromatograph analyses (B.C. Department of Agriculture Pesticide Laboratory) of 4500 ml water samples (extracted in hexane and frozen) showed that concentrations measured in the aquarium and bioassay vessels had decreased 27% after 16 h but then remained constant up to 111 h. This decrease is reflected in corrected concentrations in Fig. 1 and 2 since all behavior observations were made in the 16–92 h period after the insecticide was added to the aquarium.

Behavioral observations were conducted for each test concentration as follows: individual fish were observed for 5 min in each of eleven 2 h periods beginning at 1300 and 1500 on day 1 and at 0900, 1300, and 1500 on days 2, 3, and 4. A multiple key tally counter was used to record the following behaviors: feeding, chewing, coughing (Schaumberg et al. 1967), yawning and nipping (Hoar 1951), chasing (Hartman 1965a), displaying, displacing, vacating, flight, appeasement, flicking, and thrusting. Aggressive displays included lateral, frontal, and wigwag as described by Hartman (1965a) and Kalleberg (1958). In an interaction where one fish drifted, apparently passively, with the current causing another to vacate its station the first movement was recorded as passive displacement and the second as vacating. Any other interactions in which the movements of one fish caused another to vacate its station were recorded as chasing and flight, respectively. Appeasement (Jenkins, 1969) or submission without flight (Chapman 1962) occurred when a fish folded its fins when approached by a more dominant individual. Certain additional movements, apparently comfort behaviors, were also recorded. Flicking occurred when a fish quickly moved its head to one side and then whipped the body over to the same position. Frequently a cough and flick occurred together. Thrusting was recorded when a fish propelled itself forward with one vigorous beat of the tail, then stiffened its body into a frontal display with the dorsal fin raised. Thrusting and flicking resemble S-bending and stretching, comfort behaviors recorded for sticklebacks (Tugendhat 1960).

Friedman's two-way analysis of variance (Siegel 1956) was used to test the null hypothesis that behavioral frequency was not related to insecticide concentration. Data were cast in a two-way table with rows representing time periods and columns representing fenitrothion concentrations. A cell value for the table was the mean of the response of all surviving subjects (some mortality was observed at high insecticide concentrations when coho were pinned against screens) at a particular time period and concentration. For behaviors which varied significantly ($P < 0.05$) with fenitrothion concentration a series of Sign tests (Siegel 1956) revealed the concentration at which changes occurred. Pairs of scores used in the Sign tests were matched on the basis of time at which the observations were made.

In summary, the experiments were designed to imitate as realistically as possible the conditions which juvenile coho salmon would face when exposed to forest insecticide spraying. The absence of volitional residence and artificial feeding are obvious shortcomings.

Results and Discussion

Behavior changes in response to fenitrothion exposure were evident within 2 h. The commonest change was a marked decline in frequency at very low concentrations, as in agonistic behaviors (Fig. 1) and feeding and yawning (Fig. 2). Comfort behaviors involving locomotion (flicks and thrusts, Fig. 2) increased and then declined as concentrations increased. Coughing continued to increase up to the highest concentration tested. The apparent increase in nipping frequency at high concentrations (Fig. 1) was an observational error: at very high frequencies some coughs were mistakenly labelled nips. The generally similar results for the two control tests, one for mid-October and the other late November, suggest that seasonal behavior changes were not a complicating factor.

The insecticide-induced decrease in feeding (Fig. 2) was verified by offering extra quantities of food after the regular observation period on several occasions. Feeding behaviors increased noticeably in control tests but not in tests with the insecticide present.

In addition to the foregoing behavioral changes the stations selected by fish exposed to the insecticide tended to shift from central areas in each observation section to downstream near-surface or lateral positions. At high concentrations some fish were unable to maintain position in the current and were swept downstream and pinned against the screens. Ninety of 92 pinning incidents occurred at the two highest concentrations tested, 0.48 and 0.75 ppm. Two behavioral abnormalities were observed in the bioassays but not in the aquarium: after 5 h exposure coho swam near the surface in a head up-tail down position and their stomachs became noticeably bloated with a clear fluid. In sublethal concentrations both conditions cleared up within 48 h.

In evaluating the behavioral changes discussed below it is noteworthy that little information is as yet available on fenitrothion concentrations in streams as a result of forest spraying. Wildish et al. (1971) estimated that a 2 oz/acre application might be expected to produce a stream concentration of 0.05 ppm. Peterson and Zitko (1974) measured concentrations as high as 0.005 ppm after operational spraying. The former figure is half of the lowest concentration tested in the present study. The

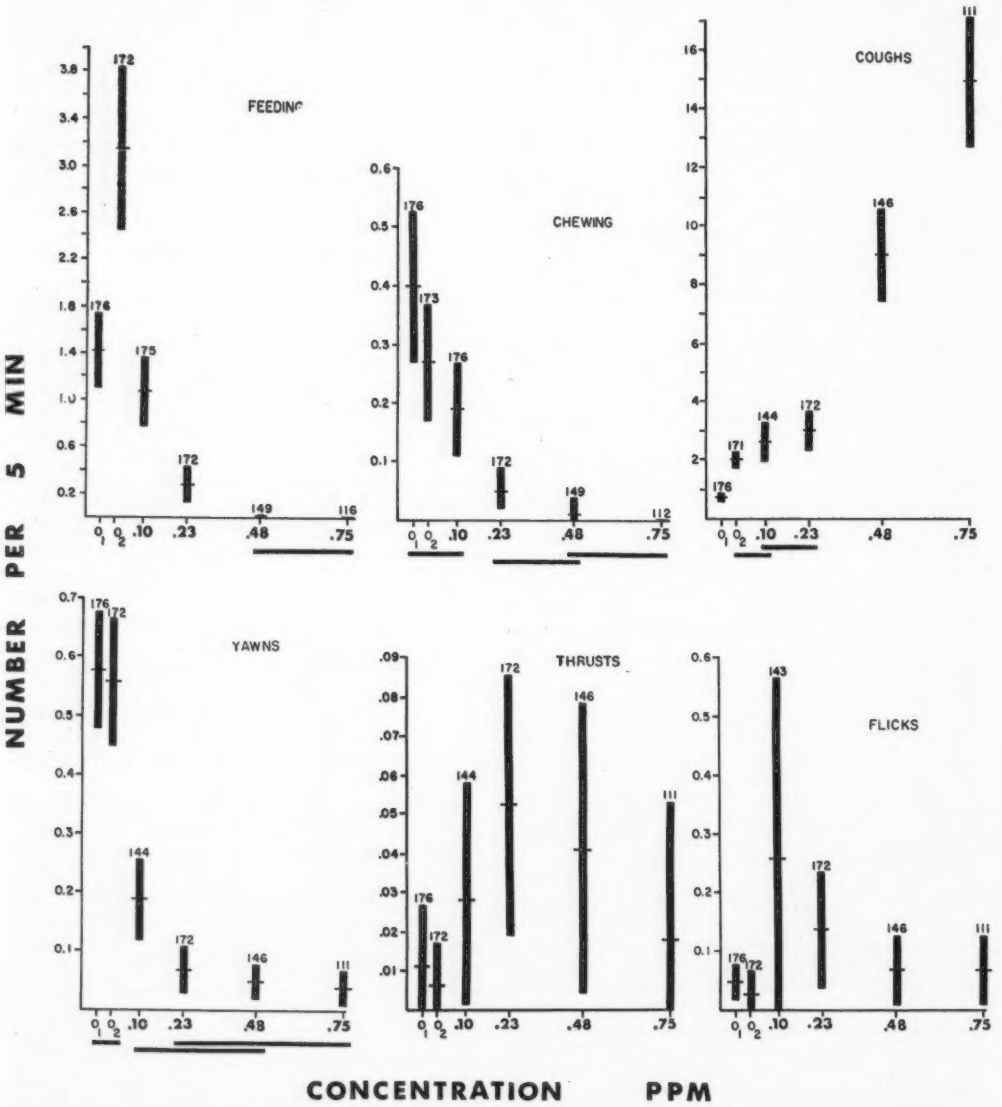

FIG. 2. Frequencies of feeding (feeding, chewing) and comfort behaviors (rest) of underyearling coho salmon at several concentrations of fenitrothion. Further details as in Fig. 1.

consumption of contaminated food (Hatfield and Riche 1970) and rapid nondietary uptake of fenitrothion may contribute to and prolong behavioral changes even though the ambient concentrations are low. In bioassays Bull (1971) found that, even in the absence of feeding, underyearling coho accumulated fenitrothion to tissue (whole fish) concentrations nearly 16 times (8.71 ppm) that of the initial concentration of the surrounding water (0.56 ppm) within 24 h.

Two functional phases of behavioral response to increasing concentrations of the insecticide can be seen. At low concentrations, 0.10 and 0.23 ppm fenitrothion, the overall reaction can be characterized as an irritant response phase. The mean frequencies of comfort behaviors increased while social and feeding activities were largely suspended. No locomotory impairment was evident. At higher concentrations, 0.48 and 0.75 ppm, definite physiological impairment (Weiss 1961; Holmstead 1959; O'Brien 1960) was reflected in the decreased frequencies of comfort behaviors involving locomotion (Fig. 2) and the inability of many fish to maintain position in the current. The onset of physiological impairment was not sharply defined but occurred in the concentration range 0.23–0.48 ppm fenitrothion.

Changes in frequency of coughing and agonistic behaviors may be useful indicators of contamination by various pollutants. Increases in coughing frequency with increasing concentration (Fig. 2) have been shown as sublethal responses also to DDT and kraft mill effluents (Servizi et al. 1969; Schaumberg et al. 1967; Walden et al. 1970). Neurophysiologically it is noteworthy that the motor pathways controlling coughing seem particularly resistant to the effects of fenitrothion. Ecologically the significance of coughing is difficult to assess. Suppression of agonistic behaviors at low concentrations (Fig. 1) confirms and extends Symons' (1973) finding that 20% of Atlantic salmon parr exposed to a fenitrothion concentration of 0.1 ppm stopped defending territories; the similar findings suggest that in the present work the effects were due mainly to fenitrothion rather than the emulsifier. The ecological implications of suppressed agonistic behaviors rest on the importance of the social organization characteristic of juvenile stream-dwelling salmonids. Kalleberg (1958) said that territoriality in juvenile salmon is a mechanism which ensures adequate food distribution. Mason and Chapman (1965) noted that, in juvenile coho, competition for space (as reflected in conventional agonistic interactions leading to a socially stable hierarchical or territorial organization or both) has been largely substituted for direct intraspecific competition for food (mostly invertebrate drift which is spatially diluted and probably limited [Mason 1969]). Presumably interruption of social organization would lead to poorer food utilization.

Feeding frequency itself was a behavior sensitive to very low levels of fenitrothion and one amenable to direct ecological assessment. Previous studies have shown that vertebrates including salmon will reject food contaminated with organophosphates (Misu et al. 1965; Symons 1973). In a salmon stream this may be important in reducing the amount of food available to feeding fish. But in addition the direct effects of fenitrothion exposure on feeding frequency are marked. Voluntary feeding dropped to very low levels at concentrations as low as 0.1 ppm (In Fig. 3 the response at 0.1 ppm should be compared with that for the second control, O_2; the abnormally low response for the first control, O_1 was due to difficulties with the constant rate feeding apparatus). Decreased feeding persisted even in the presence of large amounts of uncontaminated food.

Acknowledgments

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Effectiveness of Vancomycin Against Gaffkemia, the Bacterial Disease of Lobsters (Genus *Homarus*)

JAMES E. STEWART AND B. ARIE

Department of the Environment, Fisheries and Marine Service
Halifax Laboratory, N.S. B3J 2R3

STEWART, J. E., AND B. ARIE. 1974. Effectiveness of vancomycin against gaffkemia, the bacterial disease of lobsters (genus *Homarus*). J. Fish. Res. Board Can. 31: 1873–1879.

The antibiotics vancomycin, penicillin G, novobiocin, and oxytetracycline were examined for in vivo effectiveness against gaffkemia, the fatal bacterial infection of lobsters (genus *Homarus*) caused by *Aerococcus viridans* (var.) *homari* (formerly *Gaffkya homari*). Only vancomycin was truly effective against an established infection and then only when given at high levels (25 mg/kg lobster body weight) in the early stages of infection, prior to development of hemolymph bacterial numbers in excess of 3×10^4/ml or coincident hepatopancreatic bacterial levels of 1×10^7/g. Even massive cumulative doses (500 mg vancomycin/kg body weight), when given after establishment of maximum bacterial numbers, failed to impede the infection. Rates for the clearance of vancomycin from lobsters, established by using three different concentrations (25, 10, and 5 mg/kg body weight, respectively), were extremely slow and markedly concentration dependent. Twenty-five milligrams vancomycin/kg body weight injected prior to infection still gave a high degree of protection against challenge with the pathogen 50 days later in lobsters held at 15 C. One milligram vancomycin/kg body weight administered prior to infection gave complete protection against gaffkemia for 15 days after the antibiotic had been administered.

STEWART, J. E., AND B. ARIE. 1974. Effectiveness of vancomycin against gaffkemia, the bacterial disease of lobsters (genus *Homarus*). J. Fish. Res. Board Can. 31: 1873–1879.

Nous avons éprouvé l'efficacité des antibiotiques vancomycine, pénicilline G, novobiocine et oxytétracycline contre la gaffkémie, l'infection bactérienne fatale des homards (genre *Homarus*) causée par *Aerococcus viridans* (var.) *homari* (anciennement *Gaffkya homari*). Seule la vancomycine est véritablement efficace contre une infection établie. Et encore l'est-elle seulement lorsque administrée à forte dose (25 mg/kg de poids corporel de homard) au début de l'infection, avant que le nombre de bactéries de l'hémolymphe ne dépasse 3×10^4/ml, ou que les niveaux de bactéries dans l'hépatopancréas n'atteignent en même temps 1×10^7/g. Même des doses cumulatives massives (500 mg de vancomycine/kg de poids corporel) administrées après établissement d'un nombre maximal de bactéries sont impuissantes à arrêter l'infection. Les taux de clearance de la vancomycine des homards, établis pour trois concentrations différentes (25, 10 et 5 mg/kg de poids corporel respectivement) sont extrêmement lents et dépendent fortement de la concentration. Une injection de 25 mg de vancomycine/kg de poids corporel avant l'infection assure un haut degré de protection contre le microbe pathogène 50 jours plus tard, chez des homards maintenus à 15 C. Un mg de vancomycine/kg de poids corporel, administré avant l'infection, assure complète protection contre la gaffkémie 15 jours après administration de l'antibiotique.

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AMONG lobsters held in captivity, high mortalities periodically result from outbreaks of the fatal bacterial infection, gaffkemia. The lack of host defenses against virulent strains of the causative agent, *Aerococcus viridans* (var.) *homari* (formerly *Gaffkya homari*) has been emphasized in recent reviews (Stewart and Rabin 1970; Sindermann 1971). Thus, the development of some form of resistance through immunization or by the use of chemotherapeutic aids for protection or treatment is highly desirable. Accordingly, this report is concerned with the selection of an antibiotic having appropriate in vivo effectiveness and a determina-

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tion of the rate of elimination of the drug from the lobster.

Before proceeding with this report a brief review of the history of the changes in the name of this pathogen is appropriate and useful. The disease was described originally by Snieszko and Taylor (1947); in a subsequent report Hitchner and Snieszko (1947) proposed *Gaffkya homari* as the name for the causative agent. Recently, however, the generic term *Gaffkya* Trevisan was rejected (Judiciary Commission of the International Committee of Systematic Bacteriology 1971) and a certain amount of confusion surrounding the name of this bacterium ensued. For example, Kocur and Martinec (1965), the successful petitioners for rejection of the term *Gaffkya*, recommended that *Gaffkya homari* should be called *Pediococcus homari*, thereby following the proposal of Deibel and Niven (1960). The controversy has been resolved in the latest edition of Bergey's Manual (Buchanan and Gibbons 1974) in which the recommendations of Kelly and Evans (1974) have been followed with the result that *Gaffkya homari* is now known as *Aerococcus viridans*. Kelly and Evans (1974) also suggested that it might be desirable to separate the lobster strains from the type species, *A. viridans*, by considering the lobster strains a subspecies. We have instituted this recommendation by adding the term "*homari*" (of the lobster) following the usual nomenclatural practice of casting the term in the genitive singular case (modern Latin). Thus, the organism previously known as *Gaffkya homari* is referred to by us as *Aerococcus viridans* (var.) *homari*.

Materials and Methods

Lobsters

The large chelae of each lobster were closed with rubber bands immediately after the animals were captured locally. All lobsters (each weighing about 500 g) were held at 15 C for a minimum of 2 wk and were shown to be free of the pathogen, *A. viridans* (var.) *homari*, before being included in an experiment. The animals were not fed during the experiments. Individual shelters, which the lobsters could use at will, were provided in all tanks. In the actual experiments, the lobsters were divided into groups; one group to each tank and the animals were maintained at 15 C throughout.

Aerococcus viridans (var.) *homari*

The virulence of *A. viridans* (var.) *homari* (Rabin's strain) was maintained by continuous passage of the pathogen through lobsters. For the purpose of experimental infection standard inocula were prepared as follows: 0.2 ml of blood from the infected lobster was transferred to 50 ml trypticase soy (TS) broth (Baltimore Biological Laboratory, Baltimore), which was incubated at 28 C. The cells were harvested after 24 h and the washed cells were suspended in sterile 3% NaCl solution and standardized (0.1 optical density at 420 nm equal to approximately 5×10^6 bacteria/ml of suspension). The numbers of viable bacteria in all suspensions were determined by the method of Miles and Misra (1938) using TS agar incubated at 28 C. All dilutions were made with sterile 3% NaCl. Lobsters were infected by injecting 1.0 ml of the standardized *A. viridans* (var.) *homari* suspension/kilogram body weight into the ventral abdominal sinus of each animal; control animals received 1.0 ml/kg of sterile 3% NaCl solution. The injection site was swabbed with 70% ethanol before and after injection.

The procedure for determining bacterial numbers in hemolymph removed from lobsters challenged with *A. viridans* (var.) *homari* and treated with antibiotics included washing the bacteria twice with sterile 3% NaCl (centrifugation at 30,000 × *g*) to eliminate excess antibiotic, then resuspending the cell pack in a volume of 3% NaCl equivalent to the original sample size. Washing did not significantly affect the counts.[1]

The minimum volume of sterile distilled water required to dissolve the antibiotics was used to prepare primary solutions. These solutions were prepared for injection by further dilution in 3% NaCl sterile solution to obtain the desired concentrations.

The presence of vancomycin in the hepatopancreas and the muscle was determined as follows: 1 g of the tissue was suspended in 2 ml of sterile 3% NaCl and homogenized for 5 min at 4 C using a Lourdes blender. After centrifugation, 0.02 ml from the supernatant fluid was absorbed on a blank sensitivity disc (Sensi-Disc, Baltimore Biol. Lab., Baltimore, Md.). Vancomycin in the hemolymph was determined by placing 0.02 ml of the undiluted serum directly on the blank disc. Triplicate disc sensitivities were performed by placing the discs upon 90-mm agar plates previously seeded with 0.5 ml of a standardized suspension of *A. viridans* (var.) *homari* (5×10^6 microorganisms/ml) prepared from a TS broth culture incubated for 24 h at 28 C.

At the end of the incubation period, 24 h at 28 C, the diameters of the growth inhibition zones around the discs were measured and average values calculated. The results were compared to a standard curve prepared using known concentrations of vancomycin absorbed on blank discs as described above.

Parallel determinations on binding of the vancomycin were carried out in vitro by adding known concentra-

[1]Antibiotics: Novobiocin Sodium Salt — Albamycin, powder (The Upjohn Co. of Canada, Don Mills, Ont.); Penicillin G — Buffered penicillin G potassium salt for injection, powder (Eli Lilly and Co. [Canada] Ltd., Toronto, Ont.); Oxytetracycline hydrochloride — Terramycin, powder for intravenous injection (Pfizer Co. Ltd., Montreal, Quebec); Vancomycin — Vancocin, hydrochloride, powder for intravenous injection (Eli Lilly and Co. [Canada] Ltd., Toronto, Ont.).

tions of vancomycin to sterile hemolymph sera and hepatopancreatic homogenates and then incubating the mixture at 15 C for 1 h. The residual antibiotic was determined using the Sensi-discs as described above.

Experimental and Results

The tolerance of uninfected lobsters to penicillin G, vancomycin, oxytetracycline and novobiocin, chosen because of in vitro effectiveness (Stewart and Cornick 1967), was determined with small numbers of lobsters. This plus the preliminary in vivo screening for effectiveness against the pathogen is described briefly in the next four paragraphs and indicated that vancomycin was the most promising antibiotic, consequently it was selected for the more intensive examination.

The first step consisted of examining the tolerance of uninfected lobsters to the several antibiotics. Two of three lobsters which received 2 million International Units (IU) of penicillin G/kilogram body weight died 6 days after injection. Further trials with penicillin G in which one lobster received 80,000 and another received 400,000 IU/kg showed no apparent ill effects. Two lobsters which received 40 and 20 mg vancomycin/kg, respectively, survived unimpaired. Another two lobsters were given 22 and 11 mg oxytetracycline/kg, respectively; the one receiving the higher dose died 5 days later. Two lobsters received 7.5 and 15 mg/kg of novobiocin, respectively; the lower-dosed animal suffered only initial partial paralysis lasting for 5 min after injection but the higher-dosed lobster died 5 days later.

This information was used in subsequent trials with infected animals. Low dose levels of vancomycin, oxytetracycline, and novobiocin were injected into lobsters (three animals/treatment) 4 h after challenge with 5×10^6 *A. viridans* (var.) *homari* cells/kg body weight. The following results were obtained: Oxytetracycline at 5 mg/kg permitted a mean time to death (MTD) of 9 days, novobiocin at 7.5 mg/kg gave an MTD of 8 days while the infected controls (not treated with antibiotics) permitted an MTD of 16 days. All of these animals were shown by gram stained hemolymph smears to be massively infected. The group of lobsters receiving 20 mg/kg of vancomycin showed no signs of infection between the time of challenge and the termination of the experiment, 77 days later.

A subsequent experiment using penicillin G, vancomycin, and oxytetracycline was conducted (using three lobsters/treatment) to determine whether these antibiotics would be effective if administered 24 h after lobsters had been injected with 5×10^6 *A. viridans* (var.) *homari*/kg. The lobsters given 80,000 IU penicillin G/kg exhibited an extended MTD of 25 days, while those given 10 mg oxytetracycline/kg had an MTD of 19 days in contrast to the MTD of 12.3 days for the control lobsters injected with *A. viridans* (var.) *homari* but given no antibiotic. The infected lobsters given 25 mg/kg of vancomycin all survived, showing no signs of *A. viridans* (var.) *homari* in the blood up to the termination date, 61 days later.

Two other groups of lobsters injected with *A. viridans* (var.) *homari* were given injections of 10 mg oxytetracycline/kg in one trial at 24 and 48 h and in the other trial at 24, 48, and 72 h after infection. The MTD was extended to 52 days for the first trial and 51 days for the second.

On the basis of these preliminary trials vancomycin was selected for further studies. The results illustrated in Table 1 demonstrate that smaller doses of vancomycin (25 mg/kg) given within 48 h following infection are more effective than larger doses given later. Fifty milligrams/kilogram administered 96 h after initiation of the infection, was completely ineffective.

The data illustrated in Fig. 1 amplify these results by showing the relationship between the numbers of bacteria in the hemolymph and the effectiveness of the levels of vancomycin administered. Twenty-five milligrams vancomycin/kilogram body weight was effective in eliminating *A. viridans* (var.) *homari* up to and including a 3×10^4/ml bacterial level in the hemolymph; this same concentration administered at the 1×10^6 bacteria/ml of hemolymph level appeared to be effective initially but ultimately permitted a resurgence of the pathogen. Fifty and

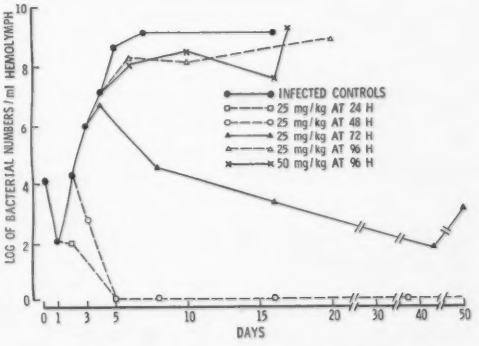

FIG. 1. Growth in control animals (not treated with vancomycin) as compared with growth of *Aerococcus viridans* (var.) *homari* in the hemolymph of lobsters held at 15 C and challenged with 2.4×10^6 bacteria kg host body weight, then treated with one dose of vancomycin at the stated intervals after infection. These are the same animals which are represented in Table 1 as Group 2 through 6.

25 mg vancomycin/kg administered at the 1×10^7 bacteria/ml hemolymph level had virtually no effect. A total of 500 mg vancomycin/kg body weight administered in five equal doses at 12-h intervals starting at the 120th h postinfection time (coincident with the completion of the logarithmic phase of growth in the hemolymph) was also ineffective (Table 1).

When vancomycin was administered prior to infection even at relatively low dose levels, protection was provided against challenge with considerable numbers of *A. viridans* (var.) *homari* (see Table 2). The trials consisted of challenging animals with the pathogen at various intervals after administration of different levels of vancomycin. In the longest trial period 25 mg vancomycin/kg gave protection against standard challenge doses of *A. viridans* (var.) *homari* up to 50 days. One milligram vancomycin/kilogram gave protection to challenge at 15 days but at 20 and 30 days only extended the

TABLE 1. Postinfective treatment of lobsters infected with *Aerococcus viridans* (var.) *homari* and held at 15 C.

No. lobsters/ group	*A. viridans* (var.) *homari* inoculum bacteria/kg body wt	Time of Vancomycin or 3% NaCl administration after injection of *A. viridans* (var.) *homari*	Vancomycin injection mg/kg body wt	MTD (days + SE) after challenge	Mean numbers of *A. viridans* (var.) *homari*/ ml hemolymph at death or termination of experiment	Observation period (*days*)
①						
3	5×10^6	4 h	20	No deaths	0	130*
Control 3	5×10^6	4 h	0‡	16±4.9	1.06×10^9	25
②						
8	2.4×10^6	24 h	25	No deaths	0	101*
Control 8	2.4×10^6	24 h	0‡	14.1±2.6	3.5×10^9	23
③						
5	2.4×10^6	48 h	25	No deaths	0	101*
Control 5	2.4×10^6	48 h	0‡	15.2±4.2	3.8×10^9	23
④						
5	2.4×10^6	72 h	25	Mechanical breakdown terminated trial on day 50	7×10^3	50
Control 5	2.4×10^6	72 h	0‡	15.2±4.2	3.8×10^9	23
⑤						
5	2.5×10^6	96 h	25	20.4±6.2	8.8×10^8	37
Control 5	2.5×10^6	96 h	0‡	10.6±3.2	6×10^9	21
⑥						
12	2.25×10^6	96 h	50	17.2±2.3	1.3×10^9	30
Control 10	2.25×10^6	96 h	0‡	13.4±2.4	4.4×10^9	25
⑦						
7	2×10^6	7 days	50	18 ±2.1	1.59×10^9	26
Control 5	2×10^6	7 days	0‡	16.2±3.7	2.68×10^9	25
⑧						
9	5.6×10^6	5 days	500**	13.6±3.8	1.4×10^9	35
Control 10	5.6×10^6	5 days	0‡	8.8±1.4	1.28×10^9	15

‡1 ml/kg 3% NaCl instead of vancomycin.
*All lobsters were found free of *A. viridans* (var.) *homari* during the observation period.
**Given in 100-mg/kg doses at 12-h intervals starting on the fifth day after challenge with *A. viridans* (var.) *homari*.

MTD. The smaller doses of vancomycin had no effect.

The foregoing was complemented by experiments in which the clearance rates of vancomycin from uninfected lobsters were determined by in vitro methods using three different concentrations (25, 10, and 5 mg/kg body wt). The results for the determinations made on hemolymph are illustrated in Fig. 2. Despite parallel and extensive examination of hepatopancreatic and muscle tissues the only tissue which gave positive indication of antibiotic activity was the hemolymph. The lower limit of detection of antibiotic activity in the hemolymph (4.5 μg/ml) was reached for the 5- and 10-mg/kg trials prior to day 15; in the 25 mg/kg trial, hemolymph from some but not all of the lobsters showed detectable amounts of antibiotic activity on day 18 and day 21; consequently, the values for those two measurement days are not reported.

In the in vitro tests carried out with hemolymph sera and hepatopancreatic homogenates to determine whether the vancomycin was bound readily by constituents of the two tissues, the residual antibiotic activity after 1 h of incubation at 15 C corresponded to the known concentrations originally added. No binding occurred under the conditions of the test.

Discussion

In lobsters held at 15 C a dose level of 25 mg vancomycin/kg body weight was effective against an *A. viridans* (var.) *homari* infection if administered within 48 h of the initiation of the infection. When the drug was administered at the same level 72 h after infection, the pathogen was controlled for an extended period (between 40 and 50 days) but was not eliminated (see Fig. 1). The same or higher dose levels given at later postinfection times had virtually no effect (see Fig. 1, Table 1); massive cumulative doses totaling 500 mg/kg initiated on the 5th postinfection day were ineffective but interestingly were well tolerated by the healthy control lobsters which received only vancomycin.

The results are more properly understood in terms of bacterial numbers in the various tissues than in terms of time alone. Assuming that the actual end point for 25 mg/kg of vancomycin effectiveness lies somewhere between 48 and 72 h at 15 C, then the equivalent hemolymph numbers would be 3×10^4 and 1×10^6/ml, respectively, still well within the logarithmic hemolymph growth phase. When the bacterial numbers in the hepatopancreas are considered, the values are considerably higher, i.e. at 48 h the hepatopancreas contains

TABLE 2. Vancomycin pretreatment and lower protective dose.[a]

No. lobsters/ group	Vancomycin injection mg/kg body wt	Day of *A. viridans* (var.) *homari* standard challenge following vancomycin treatment	MTD (days + SE) after challenge	Observation period (*days*)	*A. viridans* (var.) *homari* in hemolymph at death or termination of experiment
5	25	1	No deaths	77	0
5	25	3	No deaths	77	0
5	25	5	No deaths	77	0
5	25	10	No deaths	77	0
5	25	15	No deaths	77	0
10	25	30	No deaths	45	0
10	25	35	No deaths	45	0
5	25	50	one dead 17 days after challenge	70	one animal $>10^8$/ml 4 animals not infected
20	1.0	1	No deaths	50	0
10	1.0	10	No deaths	45	0
10	1.0	15	No deaths	30	0
5	1.0	20	40±4.6	62	Abundant
10	1.0	30	20.7±2.4	28	Abundant
20	0.1	1	15.8±1.2	24	3.1×10^9/ml
5	0.01	1	15±2.54	27	Abundant

[a]No deaths among control animals injected only with 3% NaCl. MTD for corresponding control animals injected with standard challenge dose of *A. viridans* (var.) *homari* was approximately 14 days, all with massive infections.

1×10^7 *A. viridans* (var.) *homari*/g and at 72 h about 1×10^8/g (Stewart and Arie 1973). Thus, at these times, the hepatopancreas contains the bulk of the bacteria in infected lobsters by factors of 20- to 25-fold. The heart also contains much higher bacterial numbers than the hemolymph at the same time intervals but because of its small size does not contribute greatly to the total numbers of the pathogen in the animal. Thus, to be effective, treatment of lobsters infected with *A. viridans* (var.) *homari* must come early in the infection, before the numbers exceed 1×10^7/g in the hepatopancreas or 3×10^4/ml in the hemolymph. The times at which these numbers develop in the lobsters' tissues are temperature dependent (Stewart et al. 1969); consequently tissue bacterial numbers are a far better index than a time scale could be.

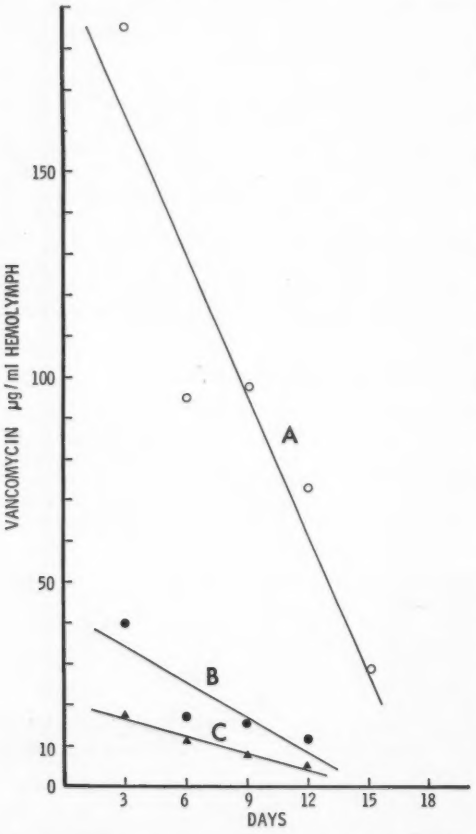

FIG. 2. Regression lines calculated for disappearance of vancomycin from the hemolymph of healthy lobsters held at 15 C. Each line represents the mean vancomycin values in hemolymph for a group of 10 lobsters. Line A (25 mg vancomycin injected/kg body weight) $Y = 196.15–11.15X$; Line B (10 mg vancomycin injected/kg body weight) $Y = 42.5–2.83X$; Line C (5 mg vancomycin injected/kg body weight) $Y = 20.75–1.37X$.

Vancomycin, when administered prior to infection even at relatively low dose levels, afforded considerable protection against challenge (see Table 2). The highest level tested, 25 mg/kg was still present in sufficient strength 50 days after injection to protect four of five lobsters challenged with a standard dose of *A. viridans* (var.) *homari*; the single death from gaffkemia, however, suggests that protection was minimal at this time and was probably less than that present 15 days after a 1-mg/kg dose. The 1-mg/kg concentrations gave complete protection for 15 days; the end point for effectiveness presumably lies somewhere between the 15- and 20-day values. These findings indicate the very slow rate at which vancomycin was cleared by the lobsters.

This was borne out by the clearance studies illustrated in Fig. 2. High concentrations in the hemolymph declined at markedly slower rates than had been shown for vancomycin clearance from blood in in vivo human studies (Kirby and Divelbiss 1957). The clearance rate in the lobster is markedly concentration dependent, with the higher concentrations declining at higher rates than the lower concentrations; thus, a clearance curve based upon the data in Fig. 2 would be hyperbolic. Rabin and Hughes (1968) have also commented on an apparently slow rate of clearance for penicillin and streptomycin in lobsters observed during a 10-day trial period.

We were unable to obtain measurements for antibiotic activity in the hepatopancreas or skeletal muscle tissue even upon injecting high levels of vancomycin into the hemolymph. Our in vitro tests did not indicate that vancomycin was readily bound by hemolymph or hepatopancreatic tissue although it is possible that some binding and concentration of vancomycin could occur in vivo. Because of the aquatic nature of the animal we were unable to determine rates of excretion for the vancomycin. Consideration of all the data, disappearance rates of the antibiotic from the hemolymph coupled with protection against challenge, and the time when the protection ends suggests that the antibiotic was being eliminated from the animal or at least converted to an inactive from.

In considering treatment of lobsters with antibiotics it is unfortunate that Furunace, the nitrofuran derivative, is not effective against *A. viridans* (var.) *homari* (Delves-Broughton 1974). Furunace, unlike vancomycin, is absorbed rapidly from the water and eliminated just as readily in both crusta-

ceans (prawns) (Delves-Broughton 1974) and flatfish (Pearse et al. 1974).

The antibiotic action of vancomycin against sensitive bacteria derives mainly from its capacity to form complexes with peptides terminating in acyl-D-alanyl-D-alanine (Nieto and Perkins 1971). These complexes are analogous to biosynthetic precursors of bacterial mucopeptides (Nieto and Perkins 1971); thus, construction of the bacterial cell wall is inhibited or impaired depending upon the vancomycin concentration used. The antibiotic will be most effective at the time of the most rapid bacterial multiplication, a fact consistent with results obtained in our study. Nakel et al. (1971) found in a comparison of *Gaffkya homari* (*A. viridans* (var.) *homari*) with two other *A. viridans* strains that most of the peptides in the *G. homari* strain cell walls did not retain the D-alanyl-D-alanine sequence at the free carboxyl termini and that none was retained in the cell wall peptides of the other *A. viridans* strains. In contrast, the peptide units of *G. homari* and *A. viridans* walls have the structures L-alanyl-γ-D-glutamyl-L-lysyl-D-alanine and L-alanyl-D-isoglutaminyl-L-lysyl-D-alanine. Since the D-alanyl-D-alanine peptides have always been found in wall peptide precursors, Nakel et al. (1971) concluded that D-alanyl-D-alanine carboxypeptidases must occur in these bacteria acting upon D-alanyl-D-alanine precursors to give the final residues actually observed. The efficacy of vancomycin antibiotic action against *A. viridans* (var.) *homari* demonstrated in this study tends to confirm this view.

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Photochemical Dechlorination of Water Supply for Fish Tanks, with Commercial Water Sterilizers

F. A. J. ARMSTRONG AND D. P. SCOTT

Department of the Environment, Fisheries and Marine Service
Freshwater Institute, Winnipeg, Man. R3T 2N6

ARMSTRONG, F. A. J., AND D. P. SCOTT. 1974. Photochemical dechlorination of water supply for fish tanks, with commercial water sterilizers. J. Fish. Res. Board Can. 31: 1881–1885.

Commercial mercury-lamp water sterilizers can be used for removal of free chlorine in water supplies, by photochemical action. The efficiency of removal decreases with increasing pH, but is little affected by temperature. It is greatest at low initial chlorine concentration. Equipment with either low or high pressure mercury vapor lamps may be used. Removal of 90% of free chlorine can be effected at about 13.0 liters min^{-1} with a sterilizer using eight low pressure lamps of 40 W, or at 50 liters min^{-1} or more with two 1200-W sterilizers with high pressure lamps in tandem. Treated water appears to be harmless to fish.

ARMSTRONG, F. A. J., AND D. P. SCOTT. 1974. Photochemical dechlorination of water supply for fish tanks, with commercial water sterilizers. J. Fish. Res. Board Can. 31: 1881–1885.

On peut employer des stérilisateurs d'eau commerciaux à lampe à mercure pour enlever, par action photochimique, le chlore libre des réservoirs d'eau. L'efficacité de l'enlèvement diminue à mesure que le pH augmente, mais n'est pas affectée par la température. On peut utiliser des appareils à lampes à vapeurs de mercure à basse aussi bien qu'à haute pression. On peut enlever 90% du chlore libre au rythme d'environ 13.0 litres min^{-1} avec un stérilisateur muni de huit lampes à basse pression de 40 watts, ou 50 litres min^{-1} ou plus avec deux stérilisateurs de 1200 watts avec lampes à haute pression en tandem. L'eau ainsi traitée semble inoffensive pour les poissons.

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CHLORINE, which is added for water disinfection, may be present in city water supplies at levels harmful to fish, and must be removed if the water is to be used for aquaria or fish-holding tanks. The usual methods of removal are by adsorption with active carbon or by reduction with sodium thiosulphate.

Free chlorine levels in city water are usually less than 1 mg Cl $liter^{-1}$, fluctuating from day to day, but sometimes exceed this level temporarily if chlorine or hypochlorite has been used for disinfection of local water mains after installation or repair. The city water supply used in the Freshwater Institute has chlorine concentrations generally in the range 0.1–0.5 mg Cl $liter^{-1}$, which is removed by metered addition of 5 mg $liter^{-1}$ sodium thiosulphate, sufficient for up to 1 mg Cl $liter^{-1}$. This quantity of thiosulphate seems to have no direct effect on fish, which may tolerate up to 1% for some time (Pyle 1960), but there have been indications that it may have interfered in some of our experiments on toxicity of mercury to fish. The toxicity of the metal appeared to be reduced, possibly by complex formation. Since further, similar experiments have been planned, we have looked for an alternative method of dechlorination, preferably without active carbon, and its attendant problems with clogging and the necessary backwashing.

When water is treated with chlorine, hypochlorite is formed, together with chloramines if ammonia is present. Blue light or ultraviolet radiation causes decomposition of hypochlorite with production of oxygen and chloride. Lewis (1912) and Spencer (1914) investigated the reaction and found that the amount of decomposition was proportional to the energy absorbed, that first order kinetics were followed, and that the temperature coefficient was small. Hancil and Smith (1971) have shown that chlorine will oxidize organic matter in wastewater exposed to ultraviolet radiation.

Equipment is commercially available which is designed for sterilization of water by ultraviolet

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radiation, and we have examined the possibility of using it for dechlorination of supplies for laboratory fish-holding systems.

The mercury vapor lamps used in such systems may be of the low or high pressure type, which differ in the quality of radiation emitted and in the power dissipated. Low pressure lamps emit substantially monochromatic radiation at 254 nm, consume typically 40 W in a tube 91.5 cm long, and run fairly cool. High pressure lamps give a line spectrum through the visible and ultraviolet, superimposed upon a continuum, and the power consumption may be 1200 W in a tube 30 cm long, which runs at high temperature. In either case the lamp is enclosed in a quartz jacket to prevent cooling by contact with water, and loss of efficiency. In both types the path of radiation through the water is kept short to avoid losses by absorption.

For the work described below we first used a number of experimental rigs with either low or high pressure lamps to measure some of the characteristics of the reaction before conducting tests on two pieces of commercial sterilization equipment.

The commercial units used were:

a) Low pressure lamp system — This used 8–91.5-cm (36-in) discharge tubes and was of 18 liters volume. Water was pumped with a plastic body centrifugal type pump, from a 200-liter polyethylene vessel through the sterilizer and then a float type flowmeter to waste. The required chlorine concentration was obtained by adding domestic bleach (5.5% available chlorine) to the water in the reservoir. Samples were taken at the inlet and outlet of the sterilizer.

b) Medium pressure lamp system — This used a single 1200-W mercury arc and was of 3 liters volume. Flow rates were higher with this system and the equipment was connected directly to the main water supply. Water was led from a regulating valve through a flow meter, and chlorine was added if required, by injection of domestic bleach with a peristaltic pump. The chlorinated water passed to two sterilizers in tandem, and then to waste. Three sampling points were provided, at the inlet to the first sterilizer, between the sterilizers, and at the outlet of the second. When the equipment was operating, a continual small stream (of about 100 ml min^{-1}) was allowed to run from each of these points.

Chlorine Determination

Samples were taken in dark glass bottles and analyzed for chlorine content by the American Public Health Association (1971) o-tolidine method.

Experiments with low and medium pressure mercury vapor lamps in experimental rigs showed that the removal of chlorine followed first order kinetics in either static conditions (Table 1) or in a flow system (Table 2). The rate constant k is given by

$$k = \frac{1}{t} \cdot 1n\left(\frac{a}{a-x}\right)$$

where a = initial chlorine concentration
$a-x$ = chlorine concentration remaining at time t

In a flow system

$$t = \frac{V}{F}$$

where V = volume of reaction chamber
F = flow rate

so that the flow rate, at which any required fraction of the original chlorine content will remain after irradiation is given by

$$k = \frac{F}{V} \cdot 1n\left(\frac{a}{a-x}\right)$$

so that, for example, removal of 90% of chlorine would require a flow rate

$$F = \frac{kV}{1n\left(\frac{100}{100-90}\right)} = \frac{kV}{2.30}$$

Tests with the low pressure lamp system and distilled water at the 1 mg Cl $liter^{-1}$ level showed that the temperature effect was quite small which is in accord

TABLE 1. Kinetics of chlorine removal by exposure to mercury vapor lamps.

T 36 lamp[a]			679A lamp[b]		
Time (*min*)	Chlorine (*mg* l^{-1})	k (min^{-1})	Time (*s*)	Chlorine (*mg* l^{-1})	k (min^{-1})
0	5.65	–	0	5.20	–
5	3.15	0.118	5	2.00	11.5
10	1.80	0.114	10	0.89	10.9
15	1.00	0.114	15	0.25	12.1
20	0.55	0.117	20	0.10	11.8
25	0.30	0.118	25	0.05	11.2
35	0.10	0.115			

[a]Lamp approximately 40 W in 3.0-liter cylinder.
[b]Lamp 450 W. Solution in silica flask at about 6 cm.

TABLE 2. Chlorine removal at different flow rates.

T 36 lamp[a]				679A lamp[b]			
Flow rate ($l\ min^{-1}$)	mg Cl l^{-1} In	mg Cl l^{-1} Out	k (min^{-1})	Flow rate ($ml\ min^{-1}$)	mg Cl l^{-1} In	mg Cl l^{-1} Out	k (min^{-1})
0.23	4.70	0.10	0.111	3.2	4.80	1.00	12.6
0.67	4.80	1.20	0.116	6.4	4.65	2.10	12.8
1.15	4.60	2.10	0.113	12.8	4.70	3.10	13.4
1.4	4.50	2.50	0.103	19.6	4.85	3.70	12.9
2.6	4.00	2.85	0.110				

[a]40-W low pressure lamp. Cylinder volume 8.0 liters, contact time $\frac{8.0}{F}$ min.

[b]450-W medium pressure lamp. Silica tube, effective volume 0.4 ml at about 6 cm from lamp. Contact time $\frac{0.4}{F}$ min.

with the increase in k of about 0.6% per degree Celsius found by Spencer (1914). With water (lightly buffered with 0.01 M phosphate) at different hydrogen ion concentrations, it was found that k decreased as pH increased, by about 10% per unit of pH.

Experiments at various flow rates with this reactor showed that dechlorination was nearly 60% more effective in tap water than in distilled water (Table 3). This may be caused by the presence in tap water of reducible organic material.

TABLE 3. Effect of flow rate on removal of chlorine with low pressure lamp sterilizer (volume 18 liters) at 10 C. Flow rate for 90% dechlorination, (from mean $k = 1.67$ for tap water), is $\frac{1.67 \times 18}{2.30} = 13.1$ liters min^{-1}.

Flow rate ($l\ min^{-1}$)	Chlorine ($mg\ Cl\ l^{-1}$) Inlet	Outlet	k (min^{-1})
	Distilled water		
3.5	0.96	0.01	0.89
6.0	1.30	0.04	1.16
8.3	1.46	0.14	1.08
9.9	1.04	0.14	1.10
11.8	1.12	0.24	1.01
15.2	1.17	0.32	1.09
	Tap water		
7.5	1.06	0.02	1.65
9.3	1.17	0.05	1.63
14.6	1.13	0.14	1.69
17.1	0.98	0.16	1.72

A series of tests in which ammonium chloride and sodium hypochlorite were added together to form monochloramine at a concentration of about 1 mg $liter^{-1}$ showed that this compound was decomposed as effectively as hypochlorite.

Two curious effects, which we cannot explain, have been observed: a) dechlorination continues after irradiation, and b) the rate constant is greater at low initial chlorine concentrations.

The delayed action (a), with a rapid decline in chlorine content immediately after irradiation, which levels off in 25 min, is shown in Fig. 1. The initial chlorine content was 0.68 mg Cl $liter^{-1}$. All our observations of final chlorine concentration were made after samples had been allowed to stand for 30 min to avoid erratic and misleading results.

The effect of initial chlorine concentration (b), is shown in Fig. 2. The rate constant is unchanged with initial chlorine concentration in the range 2–6 mg Cl $liter^{-1}$ but increases rapidly with decreasing chlorine concentration below 2 mg Cl $liter^{-1}$. This effect was found with both the low and medium pressure lamp sterilizers as will be seen below. It has the consequence that dechlorination should be more efficient, (i.e. that the overall k value should be higher) for two sterilizers operated in tandem, and we have attempted to demonstrate this with the experimental arrangement for the medium pressure lamp equipment.

The experiments were carried out with tap water, but we found some fluctuations in the chlorine content not only from day to day but from hour to hour, so that there was some difficulty in getting reproducible results. We therefore repeated our observations during a period of 6 days and report the mean values of 6 runs (Table 4). These values are

shown in the table as follows: a) upper half of table — initial values ca 0.4 mg liter^{-1}, as received in the water supply, and b) lower half of table — initial concentrations boosted to about 1 mg Cl liter^{-1} by addition of bleach solution to incoming water. The k_1 values reported are for the first reactor, and the k-total values are for the combination. The k_1 values are lower at higher initial chlorine concentrations, and there is a marked improvement (at the 1 mg Cl liter^{-1} level) in the overall k value for two reactors in tandem, which is consistent with effect (b) mentioned above. Since the chlorine levels at the outlet of the second sterilizer are quite low, k-total values are sensitive to experimental error,

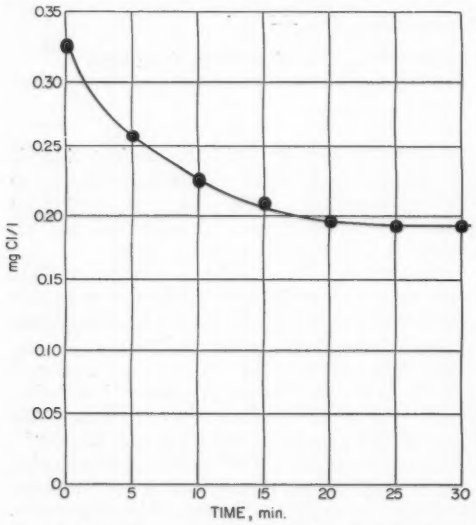

FIG. 1. Decrease in chlorine concentration after exposure to UV radiation. Medium pressure lamp sterilizer.

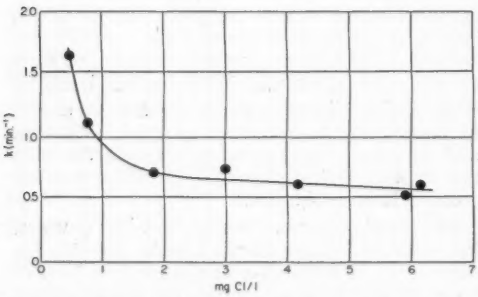

FIG. 2. Effect of chlorine level on removal of chlorine from distilled water. Low pressure lamp sterilizer at 14.6 liters min^{-1}.

TABLE 4. Effect of flow rate on removal of chlorine from tap water. Two medium pressure lamp units in tandem at 5 C. Volume of each sterilizer 3 liters. Means of six runs.

Flow rate (l min^{-1})	Chlorine (mg l^{-1}) Inlet	Outlet 1	Outlet 2	k_1 (min^{-1})	k total (min^{-1})
45.5	0.49	0.11	0.04	22.6	19.0
91.0	0.42	0.15	0.06	31.2	29.5
113.5	0.44	0.20	0.10	30.3	28.5
136.5	0.43	0.22	0.10	30.5	33.2
45.5	1.11	0.44	0.07	14.0	20.9
91.0	0.91	0.51	0.18	17.5	24.6
113.5	0.95	0.61	0.31	17.0	21.5
136.5	0.99	0.68	0.40	17.1	20.6

and it is probably unwise to read too much into the observations at low chlorine content.

These observations suggest that rate constants of 20–30 min^{-1} may be obtained with two medium pressure lamp sterilizers in tandem. The corresponding flow rates for 90% dechlorination are, therefore, 52 and 78 liters min^{-1}.

Effects on Fish

Low pressure lamp sterilizers have been in use for over 4 yr in small laboratory systems in our Institute. For obvious reasons, flow rates are governed to give about 30% safety factor at routine 0.4 mg Cl liter^{-1} concentrations, i.e. about 10 liters min^{-1}. During that time, no noticeable harmful effects have taken place except during the occasional burst of chlorine caused by poor city water system management. Some rainbow trout (*Salmo gairdneri*) have been held for over 2 yr in UV dechlorinated water; no intoxication was noted. During that time, no detectable chlorine or chloramines (o-tolidine method) were present in the fish tanks despite an average input of 0.3 mg Cl liter^{-1} and 0.08 mg chloramines liter^{-1} to the sterilizer. We do not yet have long-term observations with the medium pressure lamp equipment, but 2-wk duration exposures of guppies (*Poecilia reticulata*) in small running-water aquaria indicated no ill effects.

In our application, the sterilizers are connected to our standby power source so that outages do not affect their operation.

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Comparison of Various Vaccines for Inducing Resistance in the Lobster *Homarus americanus* to the Bacterial Infection, Gaffkemia

JAMES E. STEWART AND B. M. ZWICKER

Department of the Environment, Fisheries and Marine Service
Halifax Laboratory, Halifax, N.S. B3J 2R3

STEWART, J. E., AND B. M. ZWICKER. 1974. Comparison of various vaccines for inducing resistance in the lobster *Homarus americanus* to the bacterial infection, gaffkemia. J. Fish. Res. Board Can. 31: 1887–1892.

Various degrees of resistance to infection of lobsters *Homarus americanus* by *Aerococcus viridans* (var.) *homari* (formerly *Gaffkya homari*) were induced by vaccines prepared in different ways. Vaccines consisting of formalin-killed cells of a virulent strain of the pathogen (grown in vitro and in vivo) gave a low level of protection, i.e. the vaccinated lobsters were able to resist challenges of 200 bacteria/kg host body weight. A vaccination approach utilizing a low initial dose of the antibiotic, vancomycin, followed 24 h later by injection of 1×10^6 live pathogen cells/kg body weight produced a high degree of resistance to subsequent challenge at times considerably beyond the clearance times for the antibiotic. Fatal infections in normal lobsters are readily established with 10 pathogen cells/kg body weight whereas treatment of lobsters with the vancomycin-live pathogen combination resulted in an LD_{50} of 2×10^7 live pathogen/kg body weight. It is suggested that the relative effectiveness of the various vaccines is related directly to the concentrations of cellular components within the pathogen, possibly precursors of cell wall mucopeptides.

STEWART, J. E., AND B. M. ZWICKER. 1974. Comparison of various vaccines for inducing resistance in the lobster *Homarus americanus* to the bacterial infection, gaffkemia. J. Fish. Res. Board Can. 31: 1887–1892.

A l'aide de vaccins préparés de plusieurs façons, les auteurs ont provoqué, chez des homards (*Homarus americanus*), divers degrés de résistance à l'infection par *Aerococcus viridans* (var.) *homari* (anciennement *Gaffkya homari*). Les vaccins formés de cellules tuées à la formaline d'une lignée virulente du microorganisme pathogène (cultivé in vivo et in vitro) donnent un bas niveau de protection, i.e. les homards vaccinés peuvent résister à une attaque de 200 bactéries/kg de poids corporel de l'hôte. Une méthode de vaccination utilisant une faible dose initiale de l'antibiotique vancomycine, suivie d'une injection de 1×10^6 cellules pathogènes vivantes/kg de poids corporel, produit un haut degré de résistance contre une attaque subséquente à des temps dépassant de beaucoup les temps de clearance de l'antibiotique. Des infections fatales se produisent facilement chez des homards normaux avec 10 cellules pathogènes/kg de poids corporel, alors que le traitement de homards avec une combinaison vancomycine-microorganismes vivants résulte en une DL_{50} de 2×10^7 microorganismes vivants/kg de poids corporel. Nous croyons que l'efficacité relative des divers vaccins est directement liée à la concentration des constituants cellulaires à l'intérieur du microorganisme pathogène, possiblement précurseurs des mucopeptides de la paroi cellulaire.

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LOBSTERS (genus *Homarus*) are extremely susceptible to infection by the bacterium, *Aerococcus viridans* (var.) *homari* (formerly *Gaffkya homari*). The literature describing this fatal disease, gaffkemia, reviewed recently (Stewart and Rabin 1970; Sindermann 1971; Stewart and Arie 1974), stresses the complete lack of intrinsic host defenses against the infection. Since the bulk of the lobsters captured are held alive for appreciable periods, this disease is a continuing threat in commercial live lobster storage in addition to problems it poses for lobster farming ventures. Obviously it would be desirable to have effective measures available to cope with the disease.

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These measures should include both chemotherapeutic aids and the induction of resistance or some form of immunity.

A start has been made on these twin objectives. The antibiotic, vancomycin, has been shown to be effective when given in the early stages of the infection and preventive for appreciable periods if administered prior to infection (Stewart and Arie 1974). The second objective, the induction of a resistant or immune state, appeared much less promising until McKay and Jenkin (1969) showed that a freshwater crayfish, *Parachaeraps bicarinatus*, could be induced to increase its resistance to a moderately pathogenic pseudomonad. Prior to this, and despite many attempts with different species, induced resistance among the invertebrates to challenge with known pathogens had been demonstrated only for certain insects (Chadwick 1967). More recently Stewart and Zwicker (1974) showed that a degree of resistance against *A. viridans* (var.) *homari* could be induced in the American lobster, *Homarus americanus*, by using vaccines prepared from avirulent strains of the pathogen. In addition, Schapiro et al. (1974) in studies on the spiny lobster, *Panulirus interruptus*, showed that the considerable constitutive resistance of this animal to *A. viridans* (var.) *homari* could be enhanced by prior treatment with live avirulent strains of this microorganism.

It has become apparent that the nature of the antigen or immunogen is extremely important and that the range of materials which will elicit increased resistance in invertebrates is probably much more restricted than for vertebrates. Accordingly, we have examined vaccines prepared in different ways for their relative effectiveness in inducing resistance in the American lobster to *A. viridans* (var.) *homari*.

Materials and Methods

Lobsters — The large chelae of each lobster were closed with rubber bands immediately after the animals were captured locally. All lobsters (each weighing about 500 g) were acclimated to 15 C for a minimum of 2 wk and were shown to be free of the pathogen, *A. viridans* (var.) *homari*, before being included in an experiment. The animals were not fed during the actual experiments. Individual shelters, which the lobsters could use at will, were provided in all tanks. In the experimental work the lobsters were divided into groups; each group was maintained in a separate tank at 15 C.

Aerococcus viridans (var.) *homari* — The virulent strain used throughout was *A. viridans* (var.) *homari* (Rabin's strain). Its virulence was maintained by continuous passage of the pathogen through lobsters. For the purpose of experimental infection standard inocula were prepared as follows: 0.2 ml of blood from the infected lobster was transferred to 50 ml trypticase soy (TS) broth (Baltimore Biological Laboratory, Baltimore), which was incubated at 28 C. The cells were harvested after 24 h and the washed cells were suspended in sterile 3% NaCl solution and standardized (0.1 optical density at 420 nm equivalent to approximately 5×10^6 bacteria/ml of suspension). The numbers of viable bacteria in all suspensions were determined by the method of Miles and Misra (1938) using TS agar incubated at 28 C. All dilutions were made with sterile 3% NaCl. Lobsters were infected by injecting 1.0 ml of the standardized *A. viridans* (var.) *homari* suspension/kg body weight into the ventral abdominal sinus of each animal; control animals received 1.0 ml/kg of sterile 3% NaCl solution. The injection site was swabbed with 70% ethanol before and after injection.

Vancomycin — Vancocin (hydrochloride) powder for intravenous injection (Eli Lilly and Co. (Canada) Ltd., Toronto, Ont.) was dissolved in a minimum volume of distilled water. This primary solution was prepared for injection by further dilution in sterile 3% NaCl to obtain the desired concentrations for injection in the lobster's abdominal sinus.

Vaccines — Two preparative methods were used: 1) Washed *A. viridans* (var.) *homari* cells either from 48-h TS broths incubated at 28 C or from infected lobsters were killed with formaldehyde (Evans et al. 1968); the suspensions were adjusted to give a known number of dead bacteria in 0.9% NaCl and 0.3% (vol/vol) formaldehyde-solution. 2) Washed *A. viridans* (var.) *homari* cells suspended in 3% NaCl were exposed to gamma radiation (Co^{60} source) for 30 min (a time period determined previously as being slightly longer than required for complete killing action). The suspensions were adjusted to give 3×10^7 dead bacteria/ml in 3% NaCl.

All vaccines were checked for sterility using TS broth at 28 C. The vaccines were always administered by injection into the lobster's ventral abdominal sinus.

Experimental and Results

When deposited originally, the ATCC 10400 strain of *A. viridans* (var.) *homari* was virulent (Snieszko and Taylor 1947; Hitchner and Snieszko 1947). Over the years, however, it has apparently lost virulence since our acquisitions of this strain from the American Type Culture Collection have proved to be avirulent. Stewart and Zwicker (1974) showed that vaccines (formalin-killed) prepared from subcultures of one particular acquisition of the ATCC 10400 strain invoked resistance in the lobster to virulent strains of *A. viridans* (var.) *homari*. The maximum mean time to death we have observed upon challenging these "immunized" lobsters with large numbers of a virulent strain (2×10^6/kg body weight) was 32 days compared

with 13 days observed upon challenging untreated control animals. Unfortunately we lyophilized our subcultures of this particular ATCC 10400 strain and discontinued its active culture prior to determining the precise protective effect of the vaccines by challenging "immunized" animals with graded challenges (50% lethal dose or LD_{50}). When the lyophilized strain was returned to the TS broth culture conditions it grew well but no longer induced resistance in the lobster. Numerous trials were run with induction attempted using both live cells and vaccines (formalin-killed) prepared from it and with graded challenges ranging from 1×10^2 to 1×10^6/kg host body weight. Neither the lyophilization replicates nor new samples of the *A. viridans* (var.) *homari* ATCC 10400 strain obtained directly from the American Type Culture Collection were effective in inducing resistance. Other avirulent strains obtained in the course of our continuing surveys were examined similarly and none of these was an effective inducer of resistance. Apparently a particular subculture of the ATCC 10400 strain was unique and the feature which had made it effective in inducing resistance was lost during lyophilization.

Subsequently, attempts were made to elicit resistance by utilizing vaccines prepared from a virulent strain, *A. viridans* (var.) *homari* (Rabins' strain), grown in TS broth. Formalin-killed cells were injected and a degree of protection was conferred (Table 1). In the trials represented in Table 1B, over half of the lobsters challenged with approximately 20 or 200 bacteria/kg survived; none of the hemolymph smears or broth cultures of the hemolymph taken from the survivors during the 5-mo observation period contained the pathogen.

A time-course trial over a period of 30 days (Fig. 1) using a higher challenge dose showed that the resistance induced by this vaccine appeared within 4 days. It then declined to a minimum by day 12 where it remained approximately constant for the remainder of the period, but still considerably above the control value observed here and in other experiments.

In another trial, cells of *A. viridans* (var.) *homari* (Rabin's strain) were removed from a lobster in the midpoint of the infection and after being washed were used to prepare a vaccine (formalin-killed). This vaccine was administered at a lower level (1.6×10^6 cells/kg body weight) and conferred a higher degree of protection than that observed before when using vaccines from broth grown cells (Table 2).

A different method of preparing a vaccine from *A. viridans* (var.) *homari* (Rabin's strain) produced a lower degree of resistance overall than noted above, but had a curiously uneven effect. Three groups of 20 lobsters each received one, two, and three doses, respectively, of a vaccine prepared by killing (gamma irradiation) cells of the pathogen

TABLE 1. Effect of vaccines (formalin-killed) prepared from virulent, broth-grown *Aerococcus viridans* (var.) *homari*.[a]

No. of lobsters	Vaccine (*bacteria/kg body wt*)	Challenge *A. viridans* (var.) *homari*[a] bacteria/kg (*9 days postvaccin.*)	Lobsters surviving pathogen free	Mean time to death for gaffkemic lobsters (*days* ± SE *post challenge*)
A.				
10	Control	2.4×10^2	0	16.8±1.1
10	Control	2×10^6	0	11±0.8
10	1.8×10^9	2.4×10^2	2[b]	20±1.5
10	1.8×10^9	2×10^6	0	13±0.8
B.				
10	Control	2×10^1	0	13.1±1.3
9	1×10^9	2×10^1	5	14.7±2.1
10	1×10^9	1.8×10^2	5	23.6±4.9
10	1×10^9	2.3×10^3	0	17.6±2.1

[a]Rabin's strain.

[b]These two survivors were challenged again with 8×10^1 bacteria/kg 62 days after initial vaccination. Both survived and were then challenged with 1.6×10^2 bacteria/kg 80 days after initial vaccination. Again both survived and were shown to be free of the pathogen on final observation 136 days after initial vacination.

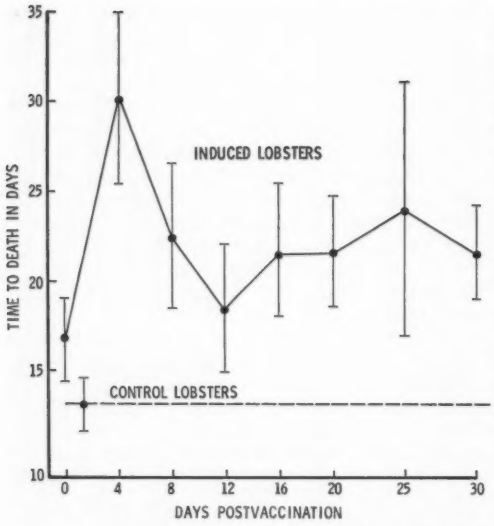

FIG. 1. Resistance induced by a vaccine prepared from TS broth-grown *Aerococcus viridans* (var.) *homari* (Rabin's strain) (formalin-killed) injected at a level of 1×10^9 cells/kg body weight. Each point represents the mean time to death plus SE for a group of 10 lobsters. Each group was challenged on the indicated postvaccination days with 1×10^3 Rabin's strain/kg body weight.

removed from a lobster in the advanced stages of the infection. The vaccine was administered at a level of 3×10^7 cells/kg body weight. Six days after the last vaccination each group of 20 lobsters was divided into four subgroups of 5 lobsters each. Each subgroup and parallel control animals received one of a graded series of challenge doses (1.3×10^3, 10^4, 10^5, or 10^6 bacteria/kg body weight). Most of the animals died with fully fledged infections in time periods which were comparable with the times to death for the control animals. Five of the "immunized" animals, however, survived and were completely free of the pathogen 31 days after challenge. These animals were divided among the various treatments, 3 had received a single dose of the vaccine and were sole survivors of the subgroups challenged with 1.3×10^3, 10^5, and 10^6 bacteria/kg. The two other survivors had received two doses of the vaccine and survived 1.3×10^3 and 1×10^5 bacteria/kg challenge doses, respectively.

The vaccine which proved to be the most successful in inducing resistance, and also the most intriguing, was a combination of the antibiotic vancomycin and the live pathogen. This approach was suggested by the studies on the efficacy of vancomycin as a therapeutic agent in this disease (Stewart and Arie 1974). In that study, groups of lobsters were given doses of vancomycin and then together with untreated control animals were challenged with the pathogen at later intervals to determine how long vancomycin was retained at effective levels by the lobster. Animals which survived the challenges were challenged again later, but without suitable corresponding controls. Surprisingly these animals survived second and third large challenge doses (10^6/kg body weight) given at times when other data indicated the vancomycin had long since been cleared. These observations were examined more critically, as shown in Table 3. Animals treated with vancomycin (1 mg/kg body weight) followed 24 h later by a single dose of the live pathogen exhibited a high degree of resistance to subsequent challenge (LD_{50} equal to 2×10^7 bacteria/kg body weight), whereas those animals given only vancomycin developed a massive infection and died. The vancomycin had a slight effect on the antibiotic control animals since the animals given vancomycin only and challenged at 20 days exhibited an increased time to death as did those challenged with the lower numbers of the pathogen on the 25th day. After bacteriological sampling, all of the survivors in this experiment (Table 3) were challenged again on the 94th day, postvancomycin treatment, with 1.6×10^6 *A. viridans* (var.) *homari* cells/kg body weight. Every animal died in the time period usually observed for untreated animals, i.e. approximately 15 days.

Discussion

Thus the American lobster which prior to treatment has no intrinsic resistance to this pathogen was shown to be capable of developing a high degree of resistance when injected with an appropriate immunogen(s). Under the usual circumstances of an infection with even small numbers of *A. viridans* (var.) *homari* the growth of the pathogen in the various tissues is so rapid that the animal is overwhelmed before a significant degree of resistance can be developed (Cornick and Stewart 1968; Stewart and Arie 1973).

These results taken together with the studies cited earlier reinforce the view expressed at the outset, i.e. the range of materials which will induce resistance or a form of immunity in crustaceans is apparently quite limited. The precise nature of the stimulant (antigen or immunogen) is not known, but the data suggest certain avenues which it might be profitable to pursue.

The effectiveness of the combination of vancomycin with the live pathogen in inducing a high degree of resistance suggests that the operative

bacterial agent(s) may be produced during cell wall synthesis. The antibiotic action of vancomycin against sensitive bacteria is derived mainly from its capacity to form complexes with peptides terminating in acyl-D-alanyl-D-alanine (Nieto and Perkins 1971). These complexes are analogous to biosynthetic precursors of bacterial mucopeptides (Nieto and Perkins 1971); thus construction of the cell wall

TABLE 2. Effect of vaccines (formalin-killed) from *A. viridans* (var.) *homari*[a] grown in vivo.

No. of lobsters	Vaccine (1.6×10^6/kg body wt)	Challenge *A. viridans* (var.) *homari*[a] bacteria/kg (*9 days postvaccin.*)	Lobsters surviving on day 83 postvaccin.	Mean time to death for gaffkemic lobsters (*days* + SE *post challenge*)
10	Control	1.6×10^2	0	14.4±1.4
10	" "	1.6×10^3	0	14.1±2.0
10	" "	1.6×10^4	0	16.4±2.1
10	" "	1.6×10^5	0	18.2±2.0
10	" "	1.6×10^6	0	13.0±1.7
10	+	1.6×10^2	10[b]	0
10	+	1.6×10^3	1[b,c]	24.5±3.4
10	+	1.6×10^4	1[b,d]	31.1±5.9
8	+	1.6×10^5	0	25.6±3.4
7	+	1.6×10^6	0	45.4±13.7

[a]Rabin's strain.
[b]Pathogen free at final observation 83 days after vaccination.
[c]Hemolymph contained 2.4×10^3 bacteria/ml at day 67 postvaccination.
[d]1.2×10^3 bacteria/ml hemolymph on day 67.

TABLE 3. Protection against *A. viridans* (var.) *homari* induced by a combination of vancomycin and live pathogen (Rabin's strain).

Treatment	Challenge time posttreatment (*days*)	Challenge *A. viridans* (var.) *homari* dose/kg	No. of lobsters surviving[a]	Deaths from gaffkemia	Mean time to death for gaffkemic lobsters (*days* + SE *post challenge*)
1 mg/kg vancomycin	20	7.6×10^2	0/10	10	20.5±2.4
"	20	7.6×10^4	0/10	10	30.2±3.1
"	20	7.6×10^6	0/10	10	23±3.3
Control	20	7.6×10^2	0/10	10	14.8±0.6
1 mg/kg vancomycin	25	1.1×10^2	0/10	10	23.2±2.8
"	25	1.1×10^4	0/10	10	26.9±3.3
"	25	1.1×10^6	0/10	10	14.8±2.6
Control	25	1.1×10^2	0/10	10	12.4±0.8
1 mg/kg vancomycin followed 24 h later by 1.6×10^6/kg live pathogen	27	0	9/10	1	49
"	27	2.0×10^3	7/10	0	0
"	27	2.0×10^4	8/10	2	30.5±0.5
"	27	2.0×10^5	7/10	0	0
"	27	2.0×10^6	5/10	5	32.0±2.1
"	27	2.0×10^7	4/10	6	22.7±3.3

[a]The hemolymph of all surviving lobsters was free of *A. viridans* (var.) *homari* on final observation 94 days postvancomycin treatment.

is inhibited or impaired directly dependent upon the concentration of vancomycin. Since the antibiotic is most effective during the stages of most rapid bacterial multiplication, the result of injecting lobsters with vancomycin followed shortly by the live pathogen should be cessation of or a greatly reduced rate of multiplication coupled with an immediate build-up of mucopeptide precursors complexed with vancomycin. The mucopeptide precursors then would appear to be possible logical candidates for the role of antigen(s).

Vaccines prepared from virulent *A. viridans* (var.) *homari* grown for 48 h in TS broth elicited a limited degree of resistance (Table 1 and Fig. 1). This suggests that the antigenic factor was present, but in low concentrations at the point where growth was interrupted. This is in accord with our hypothesis since during normal growth the mucopeptide precursors and other cell wall components would be expected to be produced at a rate matching cell wall synthesis. In addition, the bacterium at 28 C would be in the late logarithmic-early stationary growth phase where multiplication is slackening and the pool of precursors would be expected to be less. Added support for this view is offered by the results with the vaccine prepared from pathogen cells removed from a diseased lobster (Table 2). The resistance induced by this vaccine was higher than that induced by vaccines of broth grown cells (Table 1). The lobster at the midpoint of the infection would be considered a highly effective chemostat, thus the bacteria in vivo would be physiologically younger and actively dividing. They would be expected to have larger concentrations of precursors for cell walls and other cell components.

The relative effectiveness of the various vaccines is consistent with the speculative development of different concentrations of a cellular component (s). The highly effective combination of vancomycin with live pathogen suggests that the key components are probably specific peptides involved in cell wall synthesis. Presumably the immunogen(s) is the same whether produced in low concentrations by normal *A. viridans* (var.) *homari* cells or in high concentrations through a metabolic defect in an avirulent strain or blockage with a metabolic inhibitor such as vancomycin in a normal virulent strain.

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Biochemical, Physiological, and Pathological Changes in Pyridoxine-Deficient Rainbow Trout (*Salmo gairdneri*)

CHARLIE E. SMITH,[1] MYRON BRIN,[2] AND JOHN E. HALVER

Bureau of Sport Fisheries and Wildlife
Western Fish Nutrition Laboratory, Cook, Wash. 98605, USA

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Rainbow trout (*Salmo gairdneri*) fed a pyridoxine-deficient diet developed clinical signs of the deficiency after 8 wk of feeding. Erythrocyte and muscle glutamic pyruvic transaminase (GPT) concentrations were significantly depressed in deficient fish, while liver GPT was significantly elevated. Liver GPT was also elevated in starved trout suggesting the increase in enzyme concentrations was secondary to the deficiency. A normocytic, normochromic anemia, which responded favorably to pyridoxine, developed in pyridoxine-deficient trout. Microscopic lesions were found in gastrointestinal tract, kidney, liver, pancreas, and thymus of deficient fish.

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Des truites arc-en-ciel (*Salmo gairdneri*) soumises à un régime à carence de pyridoxine développent des symptômes cliniques de déficience 8 semaines après le début de l'alimentation. Les concentrations de glutamique pyruvique transaminase (GPT) sont nettement plus faibles chez les poissons déficients, alors que la GPT du foie est nettement plus élevée. La GPT du foie augmente également chez des truites privées de nourriture, ce qui donne à croire que l'élévation des concentrations de l'enzyme est un phénomène secondaire de la déficience. Il se produit, chez les truites qui ont répondu favorablement à la pyridoxine, une anémie normochromique normocytique. Nous avons observé des lésions microscopiques dans le tract gastro-intestinal, le rein, le foie, le pancréas et le thymus des poissons déficients.

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EXTENSIVE work has been done to quantitate the nutritional requirements of salmonids, especially in relation to their requirements for amino acids and vitamins (Coates and Halver 1958; Halver 1957; Halver et al. 1957; Halver and Shanks 1960; Phillips and Brockway 1957; Wolf 1951; McLaren et al. 1947; Shanks et al. 1962). In the course of these studies much experience was gained in the use of purified diets of known composition that are acceptable to fish raised under controlled conditions. Nevertheless, numerous problems in assessing the nutritional status of fish remain to be elucidated.

Description of detailed hematological and histopathological changes in salmonids with defined vitamin deficiencies are scarce (Ashley 1972; Halver et al. 1969; Poston 1967; Smith 1968; Smith and Halver 1969; Whitmore 1965; Woodall et al. 1964; Wolf 1945; Wood and Yasutake 1957). As in human and veterinary medicine, such information is useful in the clinical diagnosis of specific deficiencies. Specific enzyme reactions often have specific vitamin coenzymes, and deficiency of the vitamin generally results in reduced chemical enzyme activity. When a deficiency becomes severe, the chemical enzyme activity is reduced to the point where cellular function is impaired and appetite decreased. Finally, there is cellular pathology and death (Brin 1964a). It is inherent in this hypothesis that the biochemical defect must precede cellular death. Detection of such a defect allows identification of preclinical vitamin deficiencies. For example it is possible to determine thiamine adequacy by measuring transketolase activity (Brin 1962, 1964a,

[1]Present address: Fish Cultural Development Center, BSFW, Route 2, Box 333, Bozeman, Mont. 59715, USA.

[2]Present address: Hoffman-La Roche, Inc., Nutley, N.J. 07110, USA.

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b) in the erythrocytes of man and other mammals. Erythrocyte transaminase (aminotransferase) activity has also been used to evaluate pyridoxine adequacy in man and other mammals (Marsh et al. 1955; Brin et al. 1954, 1960; Brin 1964b; Linkswiler 1967).

In preliminary studies with rainbow trout (*S. gairdneri*) M. Brin and J. E. Halver (unpublished data) found reduced tissue transaminase activity in pyridoxine-deficient fish. Tissue transaminase activity has been reported for several fish as a liver function test (Inui 1969; Bell 1968). We describe here the effects of pyridoxine deficiency on tissue glutamic pyruvic aminotransferase (GPT) activity, and on hematological and histopathological changes observed in rainbow trout.

Materials and Methods

Yearling rainbow trout averaging 148 g in weight were divided into two groups of 50 each. One group was fed a complete test diet (CTD) (Halver 1957), the other, the same diet except that pyridoxine was omitted.

Both groups were maintained in fresh water with a constant temperature of 10 C. Fish were fed twice daily, 5 days/wk, and were weighed semimonthly.

During the 8th through 10th wk, samples of blood, liver, and muscle were analyzed for GPT activity by the method of Wroblewski and Cabaud (1957). Blood samples from 20 control and 20 pyridoxine-deficient trout were collected at the end of the 8th and 9th wk by caudal vein puncture into heparinized syringes. After the samples were centrifuged at 2000 rpm for 5 min, plasma was removed and frozen for total protein analysis. Two volumes of distilled water were then added to one volume of red blood cells (RBC). The gelation of erythrocytes (Wolf 1959) made it necessary to homogenize the sample in a glass homogenizer prior to GPT analysis.

Muscle and liver GPT were determined by homogenizing 1 g and 0.3 g, respectively, in 12 ml distilled water. These solutions were then diluted with sufficient water to give a final concentration of 17 mg/ml of muscle and 1 mg/ml of liver. One milliliter each of hemolysate or homogenate was used for each enzyme assay. All samples were incubated at 37 C for 30 min.

Hematocrits and RBC counts were determined by the method of Hesser (1960) at the start of the experiment and after 3, 6, and 9 wk. Hemoglobin concentration was determined by the cyanmethemoglobin method and total plasma protein by the biuret method (Gornall et al. 1949).

Thymus, gills, and eye as well as all internal organs were dissected from fish and preserved in Bouin's solution, dehydrated, embedded in paraffin, and sectioned at 5 μ. Tissue sections were stained routinely with hematoxylin and eosin.

At the end of 10 wk, the 10 remaining pyridoxine-deficient fish were each injected with 1 mg pyridoxine HCl, fed a recovery diet (CTD) for 3 wk, and then sacrificed. Samples of blood, liver, and muscle were analyzed for GPT and the hematology values were determined.

Results

Effects of Pyridoxine Deficiency on Trout

With an initial weight of 148 g for each group, the growth of trout fed the pyridoxine-deficient diet was normal for 8 wk, after which it declined. At the end of the 9th wk the average weight of controls was 288 g and that of the deficient trout 204 g.

Mortality first occurred in the deficient group after 8 wk reducing the group size from 50 to 10 by the 10th wk. Clinical signs were: hyperirritability, nervous disorders, erratic and rapid swimming, rapid gasping and breathing, flexing of opercles, blotchy, greenish-blue coloration, and tetany just before death. At necropsy deficient trout had pale livers, empty stomachs, and distended gallbladders.

Hematological variables were significantly decreased (hematocrit and plasma protein $P = <.05$; RBC and hemoglobin $P = <.01$) in pyridoxine-deficient fish after 9 wk (Table 1). The anemia was a normocytic, normochromic type (Hesser 1960). All hematological variables returned toward normal values after injection with 1 mg pyridoxine hydrochloride and 3 wk of recovery on the CTD (Table 1).

In pyridoxine-deficient RBC, not only was GPT activity significantly depressed ($P = <.01$) but the PLP effect (the percentage increase in enzyme activity resulting from the addition of pyridoxal phosphate (PLP) to the hemolysate sample in vitro) was significantly higher than in the controls (Table 2). This relation suggested that the GPT enzyme was less saturated with the vitamin B_6-coenzyme in deficient RBC than in control RBC. Similarly, muscle from deficient fish had significantly less GPT activity ($P = <.05$; Table 2).

Liver GPT activity was unexpectedly elevated in the deficient fish. However, it had previously been shown in rats that liver aminotransferases were highly subject to inductively increased activity when the animal was subjected to glucocorticoid exposure after fasting, total body x-irradiation, or glucocorticoid injection (Brin and McKee 1956). We suspect that a similar induction effect may have occurred in the deficient trout consequent to the inanition, and superimposed upon the effects of the onset of the vitamin deficiency.

To test this hypothesis, we compared GPT values in two groups of 10 fish each that had been starved for 8 or 16 days with GPT values in 10 control fish that had been fed in the usual manner. Although GPT activity of RBC's changed little as

a result of fasting, GPT activity of the liver in fasting fish was more than double that in the control fish at 8 days ($P = <0.01$) and was 20% greater (however, not significant statistically) than in the control fish at 16 days (Table 3). It was apparent, therefore, that the increased liver GPT activity in pyridoxine-deficient fish was secondary to the physiological effects of fasting. This conclusion was supported

TABLE 1. Hematology values (mean and standard deviation) for rainbow trout (*Salmo gairdneri*) blood.

Groups	RBC ($\times 10^6$)	Hematocrit (%)	Hemoglobin (g %)	Plasma protein (*mg/ml*)
		Pyridoxine deficient		
Initial	1.32($\pm$0.21)[a]	34.6($\pm$2.9)	8.2($\pm$.9)	32.3($\pm$4.4)[a]
9 wk	0.86($\pm$0.11)[b,c]	21.9($\pm$3.8)[b,c]	5.8($\pm$.9)[b,c]	30.6($\pm$6.5)[b]
12 wk (repleted)[b]	1.24($\pm$0.01)[c]	40.2($\pm$3.0)[c]	7.6($\pm$.7)[c]	32.9($\pm$4.6)
		Controls		
Initial	1.13($\pm$0.10)[a]	32.0($\pm$4.2)	9.1($\pm$2.2)	27.7($\pm$5.3)[a]
9 wk	1.19($\pm$0.15)[b]	26.4($\pm$2.7)[b]	7.4($\pm$.9)[b]	35.7($\pm$2.7)[b]

[a]For initial values, in comparing values for the control and different groups yielded $P = <.05$ for RBC, and for plasma protein, while for hematocrit and for hemoglobin the differences were not significantly different.

[b]For terminal 9-wk values, differences between the control and deficient groups yielded $P = <.01$ for RBC and for hemoglobin, and $P = <.05$ for hematocrit and plasma protein.

[c]Differences between the repleted group and the terminal deficient group were highly significant, $P = <.01$ for RBC, hematocrit, and hemoglobin, but not significant for plasma protein.

TABLE 2. Aminotransferase activity (mean and standard deviation) of rainbow trout tissues after 9 wk on a pyridoxine-deficient diet, and after 3 wk on a recovery diet.

Groups of trout and time of test	Aminotransferase activity		
	RBC (*units/ml*)	Liver (*units/mg*)	Muscle (*units/mg*)
Pyridoxine deficient 9 wk	20.1($\pm$7.4)[a,b,c,d]	73.1($\pm$40.0)[a,b,c]	3.3($\pm$1.9)[a,b,c]
Pyridoxine restored 3 wk	44.1($\pm$9.3)[a,c]	44.9($\pm$19.4)[a,c]	9.9($\pm$2.9)[a,c]
Controls 9 wk	43.7($\pm$10.2)[a,b,d]	62.8($\pm$27.8)[a,b]	4.3($\pm$1.0)[a,b]

[a]Units for RBC are micrograms pyruvate formed per milliliter hemolysate per hour, and for liver and muscle the units are per milligram tissue per hour.

[b]Differences between enzyme activity for control and deficient fish were highly significant for RBC ($P = <.01$), significant for muscle ($P = <.05$), and not significant for liver.

[c]Differences between enzyme activity for repleted and deficient groups were highly significant ($P = <.01$) for RBC and for muscle, and significant ($P = <.05$) for liver.

[d]Erythrocyte hemolysates were also incubated with added pyridoxal phosphate (PLP), the coenzyme form for vitamin B_6. The increment in enzyme activities (PLP effect) were as follows: controls, 22.4%; deficients, 40.0%.

TABLE 3. Effect of starvation on liver aminotransferase activity in rainbow trout (GPT units and standard deviation).

Groups of trout and no. days starvation	Aminotransferase activity	
	RBC[a] (*units/ml*)	Liver[a] (*units/mg*)
Starved		
8	46.2(±18.6)	101.9(±22.5)
16	61.5(±18.5)	57.8(±15.8)
Controls		
8	54.7(±15.0)	45.4(±5.8)
16	66.5(±20.3)	48.7(±17.9)

[a]Values for RBC were not significantly different from those of the controls, whereas those for liver were not significantly different at 16 days but highly significant at 8 days ($P = <.01$).

by the observation that GPT values of fish placed on a recovery diet in our principal experiment had returned to normal within 3 wk (Table 2).

Histopathological changes were noted in sections of kidney, liver, pancreas, intestine, pyloric caeca, and thymus from pyridoxine-deficient trout. Kidney pathology consisted mainly of cloudy swelling of proximal convoluted tubules, and a few tubules showed hydropic degeneration. Glomeruli were often shrunken, and calcium oxalate crystals were found in the kidneys of 2 of the 10 trout examined (Fig. 1).

Liver cells had mild diffuse degenerative changes consisting mainly of karyolysis and pyknosis of nuclei. The moderate glycogen and fat vacuolation normally seen in liver cells of control fish were usually absent in liver cells of deficient trout.

Sloughing of mucosal epithelium into the lumen of the intestine and pyloric caeca resulted in an accumulation of necrotic cells and mucus (Fig. 2).

Necrosis and hemorrhage were evident in pancreatic acinar cells, which are commonly found surrounding pyloric caeca and intestine (Fig. 3). Fatty necrosis was also observed in adipose tissue surrounding pyloric caeca.

Serial sections of thymus from control and deficient fish showed diffuse pyknosis of thymocyte nuclei (Fig. 4). However, measurement of the largest section from each fish with an ocular micrometer showed that thymi from deficient fish (4.4 mm) were significantly smaller ($P = <.05$) than those of controls (7.6 mm).

Adrenal cortical tissue, which normally occurs in the anterior hematopoeitic tissue of trout, was hyperplastic.

Discussion

Workers studying the vitamin requirements of trout and salmon had earlier demonstrated that pyridoxine is an essential component of the diet (Coates and Halver 1958; Halver 1957; McLaren et al. 1947; Phillips et al. 1956, 1957, 1958; Wolf 1951). Pyridoxine-deficient diets usually impaired growth within 4–6 wk and resulted in complete mortality within 8–14 wk. In our study, impaired growth and mortality were not evident until the end of 8 wk. However, fingerling trout have a greater pyridoxine and a greater protein requirement than large trout (Phillips and Livingston 1965; Mertz 1972). Thus, a high protein diet results in a more rapid onset of the deficiency (Phillips et al. 1960; C. E. Smith unpublished data). The deficiency signs we describe are typical of those observed by others.

Descriptions of hematological changes in pyridoxine-deficient fish are scarce. Anemia is one of the deficiency signs observed in chinook salmon (*Oncorhynchus tshawytscha*) (Halver 1957); however, specific effects on hematological characteristics and description of the type of anemia were lacking.

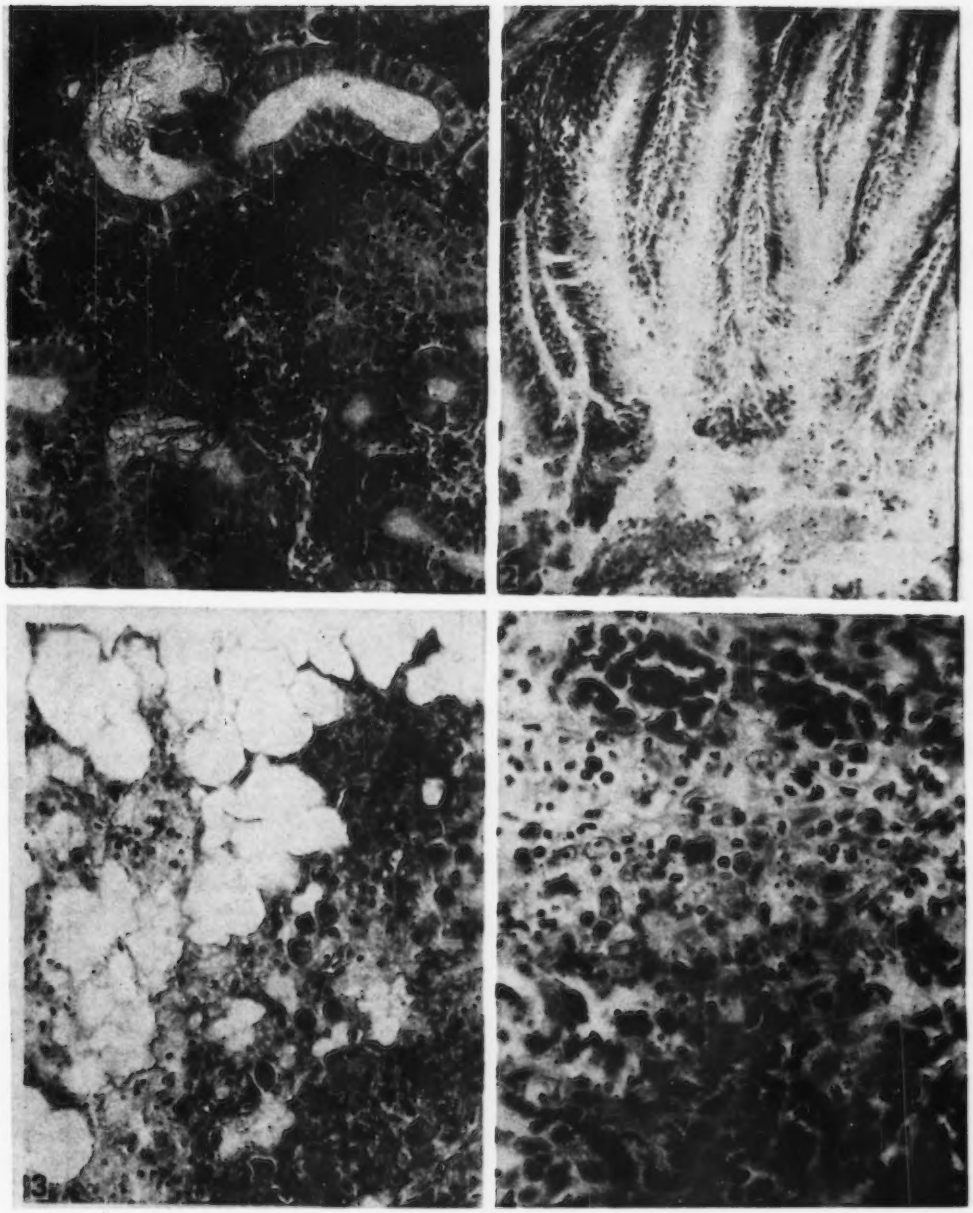
Smith (1967) did show that an anemia developed in coho salmon (*O. kisutch*) fed a diet with reduced pyridoxine content.

It was apparent from our study that pyridoxine is important for maintaining normal erythropoiesis in rainbow trout, inasmuch as the trout developed normocytic, normochromic anemia. The size of the erythrocytes and their mean corpuscular hemoglobin concentration were essentially the same as those of the controls. Anemias in pyridoxine-deficient swine, monkeys, dogs, cats, ducks, chicks, and rats were microcytic, hypochromic anemias (Shen et al. 1964) in which the size and mean corpuscular hemoglobin concentration of erythrocytes were reduced.

Since pyridoxal phosphate is the coenzyme of aminotransferases, it was significant that GPT activity was reduced in both RBC's and muscle of deficient trout. The elevation of activity in the liver may have been related to stress caused by the pyridoxine deficiency.

Pyridoxine deficiency accompanied by oxaluria has been observed in cats, rats, and rhesus monkeys (Gershoff 1964). Pyridoxine-deficient cats developed oxalate nephrocalcinosis, histologically similar to that seen in idiophatic oxalosis of man, and pyridoxine-deficient rats develop renal calcium oxalate calculi accompanied by obstructive sequelae similar to those observed in humans.

The calcium oxalate crystals observed in the pyridoxine-deficient kidneys suggest a metabolic pathway in trout common to that in higher verte-



FIGURES 1–4.

FIG. 1. Calcium oxalate crystals in convoluted tubules of kidney section from pyridoxine-deficient rainbow trout (*Salmo gairdneri*); FIG. 2. Necrosis and sloughing of mucosal epithelium in intestine of pyridoxine-deficient trout; FIG. 3. Necrosis and swelling of pancreatic acinar cells. Note also the foamy appearance of some adipose cells; FIG. 4. Karyorrhectic and pyknotic thymocytes in thymus from pyridoxine-deficient trout.

brates. Oxalate production may have been increased by the glycine in the gelatin of the diet formula. Thymus atrophy has been described in pyridoxine-deficient rats (Stoerk 1946; Combridge 1956; Butler and Morgan 1954) and chickens (Asmar et al. 1968). Stoerk (1946) showed pyknosis and karyorrhexis of thymocyte nuclei similar to what was observed in the pyridoxine-deficient rainbow trout in the present study.

Axelrod and Trakatellis (1964), who reviewed the role of pyridoxine in various immunological phenomena, showed a significant reduction in the amount of circulating antibodies as well as impairment of the delayed hypersensitivity reaction in mice. Their studies suggested that both in vivo and in vitro incorporation of amino acids into tissue protein is inhibited in pyridoxine deficiency. This decreased incorporation was accompanied by a decreased number of polysomes per unit of tissue, and in pyridoxine deficiency there was a decreased production of C_1 fragments required for biosynthesis of DNA and RNA, particularly the messenger RNA fraction. These observations link the role of pyridoxine in the immune process to its ability to catalyze the biosynthesis of nucleic acids required for both cellular proliferation and synthesis of specific immune proteins.

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Early Development and Distribution of the Flathead Sole (*Hippoglossoides elassodon*)

D. F. ALDERDICE AND C. R. FORRESTER

Department of the Environment, Fisheries and Marine Service
Pacific Biological Station, Nanaimo, B.C. V9R 5K6

ALDERDICE, D. F., AND C. R. FORRESTER. 1974. Early development and distribution of the flathead sole (*Hippoglossoides elassodon*). J. Fish. Res. Board Can. 31: 1899–1918.

Laboratory studies were conducted on eggs from a southern British Columbia population of *Hippoglossoides elassodon*. Salinity of neutral buoyancy of eggs decreased from about 25‰ shortly after fertilization to 17–18‰ at hatching. The incubation period ranged from 20.9 days (2.36–4.0 C) to 7.2 days (10.65 C). At temperatures of 5.5 C or lower and salinities of 19‰ or lower, larvae tended to hatch at a less advanced stage of development. Mean length of newly hatching larvae increased in successive periods throughout the hatching interval. At mean hatching time, larval length varied from 3.2 mm (15‰, 3.0 C) to 6.3 mm (25‰, 6 C). Larvae from eggs incubated over the range of test conditions (10.42–39.58‰, 2.36–10.65 C) reached a length of 6.9–7.2 mm at yolk exhaustion. Hatching success was highest in trials at 25‰, 6 and 7 C; maximum percent viable hatch is predicted at incubation conditions of 29.16‰, 7.38 C. Posthatching larval survival appeared normal at salinities of about 20–37‰ and temperatures of about 5.5 to >10.65 C. Extensive early larval mortality occurred at salinities of 19‰ or less and at temperatures below 5.5 C. Incipient low lethal temperature levels are estimated to be near 3.5–4.0 C for incubating eggs and 4.0–4.5 C for larvae.

Egg and larval characteristics of the experimental material were compared with similar data from the literature on North Pacific Ocean *Hippoglossoides*. A number of previous distinctions made between eggs and larvae of *H. elassodon*, *H. robustus*, and *H. dubius* require further validation owing to the possible confounding of genetic and environmental influences on the attributes studied. Distribution and abundance of *H. elassodon* over its North American range is discussed on the basis of the evidence gathered.

ALDERDICE, D. F., AND C. R. FORRESTER. 1974. Early development and distribution of the flathead sole (*Hippoglossoides elassodon*). J. Fish. Res. Board Can. 31: 1899–1918.

Nous avons effectué en laboratoire des études sur les oeufs d'une population d'*Hippoglossoides elassodon* du sud de la Colombie-Britannique. La salinité du point neutre de flottabilité des oeufs passe d'environ 25‰ peu après la fécondation à 17–18‰ à l'éclosion. La période d'incubation varie entre 20.9 jours (2.36–4.0 C) et 7.2 jours (10.65 C). A des températures de 5.5 C ou moins et à des salinités de 19‰ ou moins, les larves ont tendance à éclore à un stade de développement moins avancé. La longueur moyenne des larves nouvellement écloses augmente au cours des périodes successives, pendant toute la durée de l'éclosion. A la période moyenne d'éclosion, la longueur des larves varie entre 3.2 mm (15‰, 3.0 C) et 6.3 mm (25‰, 6 C). Les larves provenant d'oeufs incubés dans la gamme de conditions des essais (10.42–39.58‰, 2.36–10.65 C) atteignent une longueur de 6.9–7.2 mm au moment de l'épuisement du vitellus. L'éclosion a le plus de succès dans les essais à 25‰, 6 et 7 C; nous prédisons que le pourcentage maximal d'éclosion viable se trouve dans des conditions d'incubation de 29.16‰, 7.38 C. La survie des larves après l'éclosion semble normale à des salinités d'environ 20–37‰ et à des températures d'environ 5.5 à >10.65 C. De fortes mortalités, au début du stade larvaire, se produisent à des salinités de 19‰ ou moins et à des températures inférieures à 5.5 C. On estime à environ 3.5–4.0 C les niveaux inférieurs de température qui commencent à causer la mort des oeufs en voie d'incubation et à 4.0–4.5 C celle des larves.

Nous avons comparé les caractéristiques des oeufs et des larves du matériel expérimental avec des données similaires publiées sur *Hippoglossoides* du Pacifique Nord. Bien

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des distinctions établies antérieurement entre les oeufs et les larves de *H. elassodon*, *H. robustus* et *H. dubius* demandent une validation plus poussée, à cause de la confusion possible des influences génétiques et environnementales sur les attributs échantillonnés. A la lumière des renseignements recueillis, nous examinons la distribution et l'abondance de *H. elassodon* dans son aire nord-américaine.

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INFORMATION on distribution and early development of the flathead sole (*Hippoglossoides elassodon*: family Pleuronectidae) in North Pacific and Alaskan waters is somewhat fragmentary. The species is reported to occur around the rim of the North Pacific Ocean from Point Reyes, northern California (38°N, 123°W) (Hart 1973), northward through the Gulf of Alaska and across the Bering Sea (Fig. 1). In Asian waters it extends from southern Anadyr Gulf (62°N, 180°E) southward to Paramushir Island (50°N, 156°E) (Pertseva-Ostroumova 1961), along the Okhotsk Sea coast of Kamchatka to about 56°N lat., in the northwestern Sea of Okhotsk, in Tartar Strait, and on the east coast of Sakhalin (Rass and Zheltenkova 1948; Pertseva-Ostroumova 1961; Musienko 1970).

However, distribution in the Bering Sea and in Asian waters is somewhat uncertain because of an overlap in meristic characters between *H. elassodon*, *H. robustus*, and *H. dubius* (Wilimovsky et al. 1967). There is no uniform opinion on the taxonomic position of the three forms; some authors consider them to be subspecies of *H. elassodon* (Moiseev 1953). Nevertheless only *H. elassodon* (Norman 1934) has been recorded in North American waters south of the Alaskan Peninsula, including Canadian waters.

The current laboratory study was conducted on eggs from a spawning population of flathead sole in southern British Columbia waters. This study was part of a general investigation of factors affecting survival of eggs and larvae of commercially important marine species. The effects of various salinity–temperature incubation conditions on early development are described; some characteristics of the eggs and larvae are documented and compared with those of *H. robustus* and *H. dubius*. Because of the paucity of field information, some inferences regarding distribution and relative abundance are drawn from the experimental data.

Materials and Methods

Live flathead sole were captured by trawl net (March 27, 1968; April 1, 1969) in depths of 55–64 m on a spawning ground off Boat Harbour, Strait of Georgia (49°06′N, 123°46′W). Eggs were stripped from live fish approximately 3 h following capture and fertilized in sea water of 26.9–28.4‰.

Fertilized eggs were dispensed volumetrically into incubators (Alderdice and Velsen 1968) and held throughout development at predetermined salinity–temperature levels. Each incubator held four subsamples of eggs (mean, 152; range, 95–227). The experiments utilized a 13-point factorial design with replicated center point.

Salt water was prepared by making natural sea water (27–29‰) up to 40‰ with a synthetic seawater salt mixture, followed by dilution with fresh water to the required salinity. Water in the incubation tanks was renewed every 4 days. Standard errors associated with the predetermined test conditions (see Table 1) averaged ±0.02‰ and ±0.02 C over the experimental period.

Measurements of egg size and buoyancy were made at various times throughout the incubation period. The incubation period is defined as the interval between fertilization and 50% hatch. Measurements of total length (including caudal finfold) were obtained from samples of 10–15 newly hatched larvae at 8–12-h intervals for each incubation trial. Yolk-sac size was measured as the anteroposterior length of the yolk sac. Measurements were made with microscope and ocular micrometer on larvae anesthetized in a 1:1 solution of 1:15,000 M.S. 222 and salt water from the appropriate incubation tank. Results of the incubation trials are expressed in terms of rate of development (percent/day $= t/100$; t = incubation period, days), mean length of newly hatched larvae, percentage total hatch, and percentage viable hatch. Viable larvae were considered as those without visible physical deformities. In most trials posthatching growth was determined until yolk exhaustion and death of the unfed larvae.

Results of the incubation trials were subjected to response surface analysis (Lindsey et al. 1970) using second-order polynomials as models (Alderdice and Forrester 1971). Analyses were conducted with a linear model

$$Y = b_0x_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2$$

and a nonlinear model

$$Y^\gamma = b_0x_0 + b_1x_1^{\alpha_1} + b_2x_2^{\alpha_2} + b_{11}x_1^{2\alpha_1} + b_{22}x_2^{2\alpha_2} + b_{12}x_1^{\alpha_1}x_2^{\alpha_2}$$

where Y = response; x_1, x_2 = salinity (‰) and temperature (C), respectively; and γ, α_1, α_2 are parameters estimated by maximum likelihood procedures. Analyses were completed using the latter model if, by likelihood inference, it provided an improved fit of the response surface to the data when compared with that provided by the linear model.

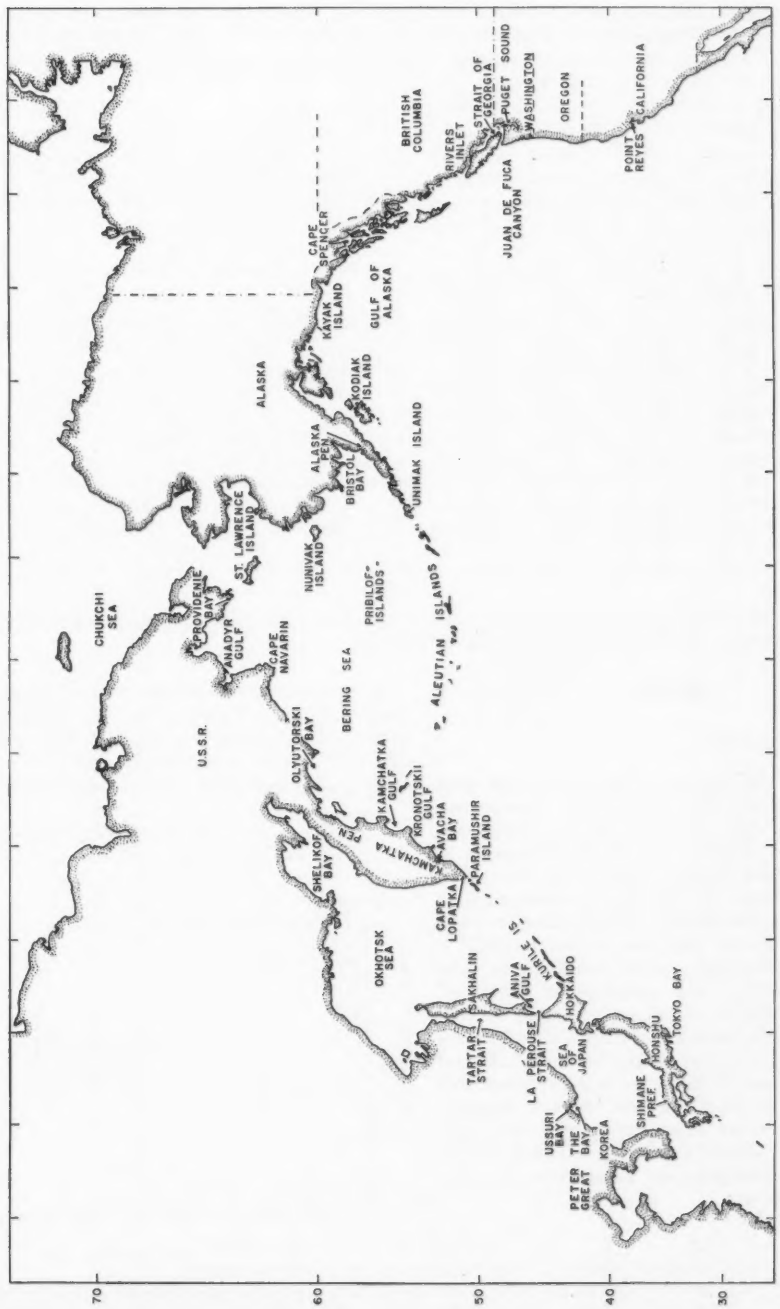

FIG. 1. North Pacific Ocean and geographic locations referred to in the text.

TABLE 1. Length of incubation period, rate of development, and mean total length of larvae at mean hatching time for flathead sole (*Hippoglossoides elassodon*) eggs incubated at various salinity–temperature conditions.

Trial	Test conditions[a] Salinity (‰)	Temp (C)	Incubation period (h)	Rate of development (%/day)	Larval size at mean hatching time (mm)	Trial	Test conditions[a] Salinity (‰)	Temp (C)	Incubation period (h)	Rate of development (%/day)	Larval size at mean hatching time (mm)
	1968						*1969*				
1	15.00	3.00	439.1	5.47	3.23	1	13.00	4.00	502.3	4.78	5.95
2	15.00	9.00	243.4	9.86	5.88	2	13.00	10.00	216.4	11.09	5.63
3	35.00	3.00	435.7	5.51	4.09	3	37.00	4.00	403.0	5.96	4.20
4	35.00	9.00	236.0	10.17	5.93	4	37.00	10.00	220.6	10.87	5.85
5	25.00	6.00	433.9	5.53	6.27	5	25.00	7.00	232.0	10.34	3.86
6	25.00	6.00	303.2	7.92	5.28	6	25.00	7.00	270.5	8.87	5.10
7	12.85	6.00	398.8	6.02	5.97	7	10.42	7.00	294.1	8.16	4.89
8	37.15	6.00	332.0	7.23	5.60	8	39.58	7.00	260.0	9.07	4.50
9	25.00	2.36	502.2	4.78	3.32	9	25.00	3.36	435.0	5.52	3.80
10	25.00	9.65	203.0	11.82	5.57	10	25.00	10.65	172.1	13.95	5.11
11	20.00	4.50	362.2	6.63	4.55	11	19.00	5.50	335.3	7.16	4.67
12	20.00	7.50	283.5	8.47	5.74	12	19.00	8.50	243.6	9.85	5.29
13	30.00	4.50	297.0	8.08	3.53	13	31.00	5.50	353.7	6.79	3.68
14	30.00	7.50	303.7	7.90	5.29	14	31.00	8.50	239.4	10.03	5.27

[a]Levels calculated from the experimental design employed are used throughout the text. Actual levels achieved were maintained with standard errors of ±0.02‰, ±0.02 C.

Results

EGG CHARACTERISTICS

Egg size — Unfertilized flathead sole eggs were spherical, nonadhesive, and almost transparent. One sample of eggs (N = 50) had an average diameter of 1.31 mm, 15 min after fertilization in water of 26.92‰. Intake of water and increase in egg diameter began shortly after fertilization. Rate of increase in egg diameter followed a characteristic pattern (Fig. 2) but final size achieved (18–25 h following fertilization) varied considerably. The following sources of size variation are compared.

1. In 12 different salinity–temperature incubation media, and between 114 and 286 h after fertilization, mean egg diameter ranged between 2.16 and 2.69 mm. In most of these, variation in size within samples was as great as that between samples. However, the mean diameters suggest that eggs are largest at salinities between 20 and 27.5‰ and at lower temperatures. These data are summarized as follows:

Temp (C)	<6	6	>6
Egg diam (mm)	2.51	2.31	2.28
Salinity (‰)	<20	20–27.5	>27.5
Egg diam (mm)	2.34	2.46	2.30

2. A reciprocal cross was made between three male and three female sole. The resulting nine groups of eggs were incubated in 25‰ sea water at 6 C in the standard manner. Initial (1.9 h after fertilization) and final (41.0 h after fertilization)

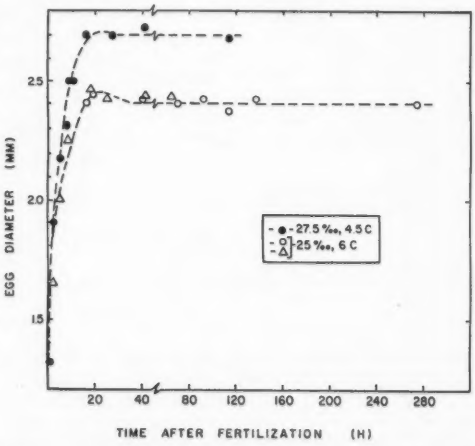

FIG. 2. Increase in egg diameter following fertilization.

egg diameters (mm) were as follows:

	Parent fish TL (♂)	Mean egg diam initial/final (*mm*) ♀1	♀2	♀3
♂1	20.8	1.64/2.53	1.63/2.62	1.88/2.88
♂2	24.5	1.62/2.47	1.63/2.60	1.90/2.96
♂3	24.5	1.70/1.97	1.66/2.36	1.90/2.89
Parent fish TL (*cm*) ♀		31.0	33.4	39.2

Variations in egg diameter within the nine groups indicated that the samples could have been drawn from the same population. However, analysis of variance suggested that heterogeneity among the final (41 h) egg diameters is associated with the individual females from which the eggs were obtained.

In general the data indicate that egg size varies with respect to 1) time following fertilization, 2) incubation conditions, and 3) source of the eggs. In all groups examined, the range of mean egg diameter 41 h or more after fertilization was 1.97–2.96 mm, with a general mean of 2.48 mm. The yolk diameter was more constant in size. When spherical in form, prior to lifting of the tail bud from the yolk, the yolk averaged 1.52 mm in diameter (range, 1.47–1.55 mm).

Salinity of neutral buoyancy. Determinations were made largely with one group of eggs incubated at 25‰, 6 C (Fig. 3). Egg density decreased from 1.0198 shortly after fertilization, to 1.0155 shortly before hatching; density of newly hatched larvae was 1.0141. The data indicate that newly spawned eggs would be buoyant in sea water of 25–27‰ or greater; newly hatched larvae would be buoyant in salinities of 17–18‰ or greater. Figure 3 suggests that egg density may be slightly greater at higher temperatures. The possibility is supported by the previously mentioned observation that egg diameters were smaller at higher temperatures. Presumably this relation could arise through the intake of less water by eggs at higher temperatures, and development of a smaller perivitelline space. Such events

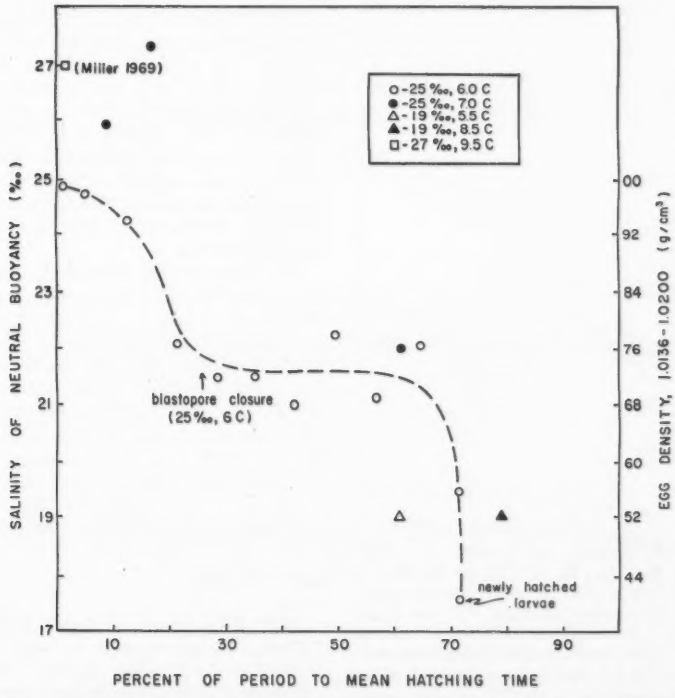

FIG. 3. Changes in salinity of neutral buoyancy in relation to percentage of incubation period completed. A trend line (fitted by eye) is indicated for eggs incubated at 25‰, 6 C.

probably would result in a smaller, denser egg with a higher salinity of neutral buoyancy.

Rate of Development

Length of the incubation period varied between 7.2 days (25‰, 10.65 C) and 20.9 days (25‰, 2.36 C; 13‰, 4 C) (Table 1). Analysis of variance indicated that temperature was the only factor with a significant effect on rate of development. Length of the hatching interval appeared to be independent of salinity and described a linear relation with temperature from approximately 90 h at 10.65 C to 190 h at 2.36 C.

Considerable variation occurred in rates of egg development at given incubation temperatures (Fig. 4), which could be related to one or more of the following:

1. *Low salinity effect* — Incubating eggs tended to lose turgor in the latter part of the incubation period at salinities of 19‰ or lower. Larvae tended to emerge tail first from the ruptured capsule at salinities below 19‰ and were less advanced in development (see Fig. 5a). Larvae were less active than at higher salinities and many remained in the ruptured capsule for 12–24 h prior to full emergence. As larvae were not considered to have "hatched" until they were free of the capsule, the incubation period in some instances is lengthened arbitrarily. Hence, rate of development, as measured, is correspondingly reduced (e.g. Table 1, 1968 — 12.85‰, 6 C; 1969 — 13‰, 4 C).

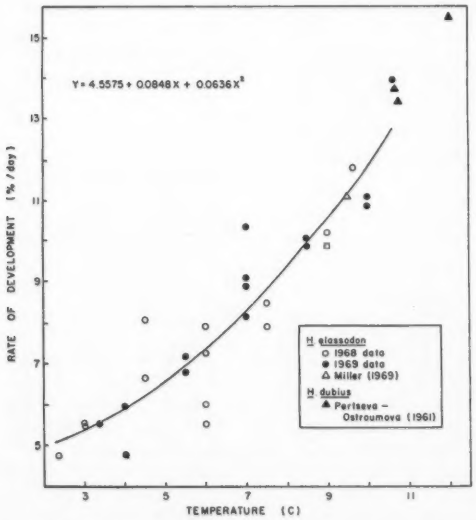

FIG. 4. Rate of development of flathead sole (*Hippoglossoides elassodon*) eggs in relation to incubation temperature. Multiple points at given temperatures represent the trials at various salinities. The trend line is fitted according to the equation shown in the figure. The points for *H. dubius* were obtained by adding one-half the hatching interval (first to last emergence) to be expected for *H. elassodon* at 10 C from the current study (45 h) to Perseva-Ostroumova's listed incubation periods to first hatch (her table 5).

2. *Low temperature effect* — Incubating eggs tended to lose turgor in the latter part of the incubation period at and below temperatures of 5.5 C. There was an apparent loss of fluid from the perivitelline space, although capsules remained intact. Larvae tended to emerge at a less advanced stage of development, at a smaller size, and with greater yolk reserves (Fig. 5a, 6).

3. *Egg density effect* — Eggs were susceptible to capsule damage in the incubators in relation to their density and the density of the incubation medium. This relation varied both with stage of egg development in each trial and with the incubation conditions of the various trials. Capsule damage was minimal or absent in high and low salinities where eggs were perfused in a direction counter to their floating or sinking tendencies. Eggs were more susceptible in salinities of 20–30‰ and at those times when eggs were near neutral buoyancy with respect to the salinity of incubation. In addition, those eggs that lost turgor at low salinities and temperatures were more susceptible to capsule damage.

The three sources of variation described tended to increase the error associated with determination of time of maximum hatching frequency. Consequently the error is reflected in estimates of rate of development, as well as larval length and yolk-sac size at mean hatching time. However, neither capsule damage nor loss of egg turgor accounted for the tendency of eggs in some trials to hatch at a less advanced stage of development; this tendency was concluded to be a natural response of the eggs to low incubation salinities (below 19‰) and in particular to low temperatures (5.5 C or lower).

Size of Larvae

Mean total length of newly hatched larvae increased throughout the hatching interval in all incubation trials (Fig. 7) (0.5 mm/day at lower temperatures and 1 mm/day at higher temperatures).

Larval size at mean hatching time (Table 1), calculated from the distributions of mean total

length at hatching (e.g. Fig. 7), ranged from 3.2 mm (15‰, 3.0 C) to 6.3 mm (25‰, 6 C). Analysis of variance indicated that only temperature had a significant effect on larval size. Larval size at mean hatching time tended to increase at higher temperatures, reaching a maximum at or above 6 C (Fig. 6).

Hatching Success

Total hatch and viable hatch were highest (>90%) in trials at 25‰, 6 C and 25‰, 7 C (Table 2). Survival to hatching was high in most trials, however, and averaged about 75% over all incubation conditions examined.

Analysis of variance indicated that treatment (salinity, temperature) effects explain a large portion of the variance associated with total hatch. A regression surface was fitted to the data on the basis of the nonlinear polynomial

$$Y^{2.048} = -9.554 \times 10^5 + 1.494 \times 10^6 x_1^{0.074} + 1.074 \times 10^4 x_2^{0.767} - 5.972 \times 10^5 x_1^{0.148} - 1.511 \times 10^3 x_2^{1.534} + 2.507 \times 10^3 x_1^{0.074} x_2^{0.767}$$

where $Y^{2.048}$ = percentage total hatch in terms of Y^{Y}. Maximum total hatch is calculated as 94.50% at the center of the surface, S, at or near 22.52‰,

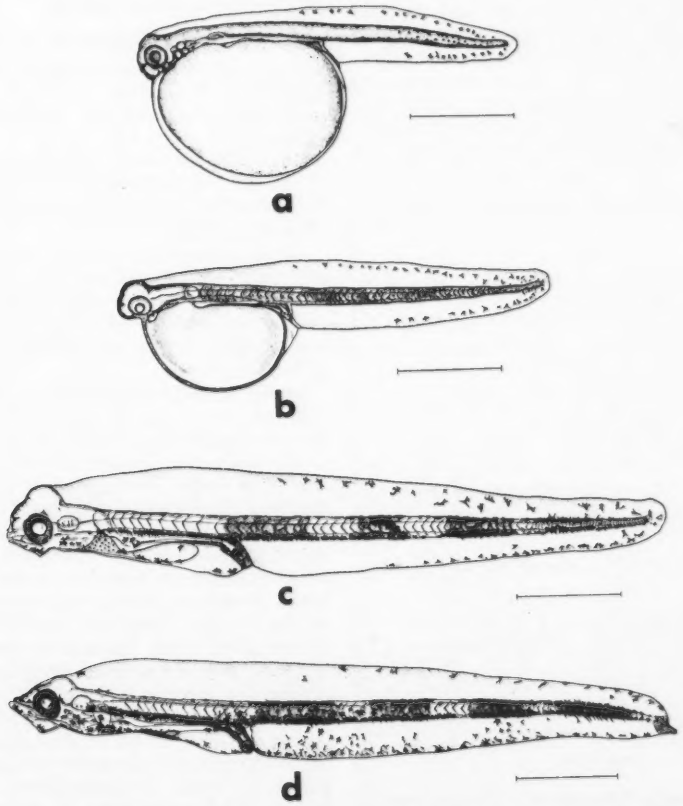

FIG. 5. Flathead sole larvae. Camera lucida drawings from photographs of live specimens. Scale shown = 1 mm. Incubation conditions: a, 15‰, 3 C — newly hatched larvae typical of those appearing to hatch at an early stage of development; b, 25‰, 9.65 C — typical newly hatched "normal" larva; c, 25‰, 9 C — 2-day-old larva; d, 25‰, 9.65 C — 10-day-old larva.

7.31 C (Fig. 8). Near-maximum total hatch (90% or greater) is predicted at salinities of 14–35‰ and temperatures of 5.5–9.0 C. No interaction between salinity and temperature is indicated with respect to percentage total hatch.

The relation between percentage viable hatch and salinity–temperature conditions of incubation was considered in the same manner as for total hatch. The nonlinear model is

$$Y^{1.081} = -584.190 + 2.616x_1^{1.263} + 451.344x_2^{0.507} - 0.020x_1^{2.526} - 83.252x_2^{1.014} + 0.100x_1^{1.263}x_2^{0.507}$$

where $Y^{1.081}$ = percentage viable hatch in terms of Y^{γ}. Maximum viable hatch is calculated as 90.14% at the center of the surface, at or near 29.16‰, 7.38 C (Fig. 9). No salinity–temperature interaction is indicated with respect to percentage viable hatch.

The components of the centers of maximum esponse at S are not determined with equal precision. The ranges of plausible salinity and temperature values, within which the calculated centers at S are maximum likelihood estimates, are approximately as follows:

	Total hatch	Viable hatch
Salinity (‰)	15–37	28–32
Temp (C)	7.0–7.5	7.0–7.5

Total or viable hatches of 50% or greater (Fig. 8, 9) are predicted for most of the range of incubation conditions investigated, and perhaps also at temperatures several degrees higher than the maximum considered (10.65 C). Near-maximum viable hatch (90% or greater) is estimated to occur within a narrow range of about 28–30‰ and 7.0–7.5 C.

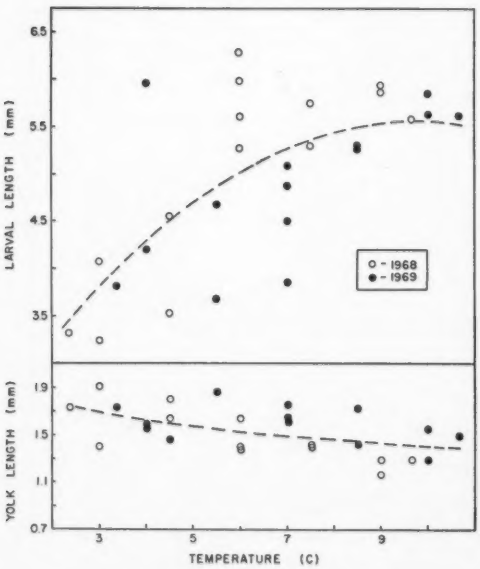

FIG. 6. Total length of flathead sole larvae and anteroposterior yolk-sac length in relation to incubation temperature. Both measures are estimated at the mean hatching time from samples taken at 12-h intervals throughout the hatching period. Multiple points at each temperature represent the trials at various salinities. The trend lines are fitted by regression analysis.

POSTHATCHING DEVELOPMENT

Samples of newly hatched larvae from most trials were put into separate containers, returned to their appropriate incubation conditions and observed until yolk exhaustion and death from starvation (Table 3).

Larvae initially were inactive; they floated passively, yolk sac upward, in those salinities in which they were buoyant. Such larvae could swim rapidly when disturbed.

Larvae ultimately became continuously active and swam with the yolk sac in a ventral position. Feeding responses increased, typical of those described by Rosenthal (1970); prey-catching behavior was exhibited toward air bubbles with a diameter of 0.08–0.24 mm.

Newly hatched larvae had a row of stellate melanophores at the margin of the finfold. These extended dorsally from above the posterior margin of the hindgut and peripherally around the caudal area to a ventral location about midway between the tip of the tail and the hindgut (Fig. 5a, b). Subsequently a diffuse and regular network of inconspicuous melanophores appeared, overlying the trunk musculature posterior to the hindgut. Four bands of chromatophores then developed posterior to the hindgut. These consisted of melanophores overlying the trunk musculature and a coincident band of yellowish-gold xanthophores. The band around the urostyle and the two adjacent bands developed first, followed by appearance of the fourth, posterior to the hindgut (Fig. 5c, d). Prior to the appearance of the anterior tailband of melanophores, a band of xanthophores formed in its presumptive location (Fig. 5). Miller (1969) noted the same sequence of events with his larvae. Xanthophores in the anterior band frequently originated above or slightly posterior to the hindgut (Fig. 5c, d), while the melanophores of the band originated nearer the fifth myotome posterior to the hindgut.

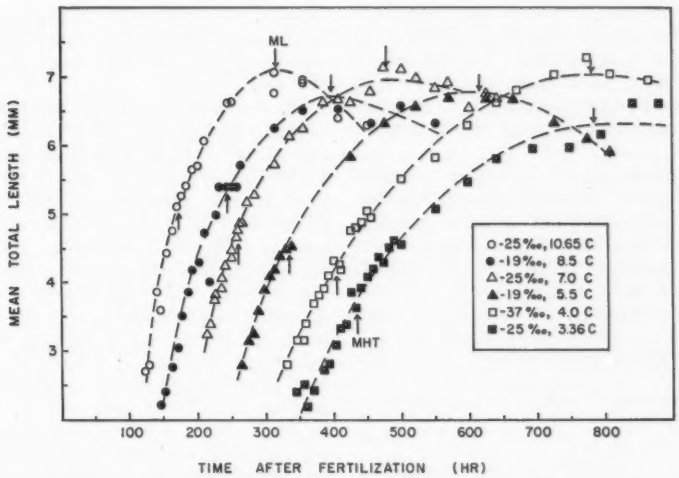

FIG. 7. Larval length in the hatching and posthatching periods (see Table 1, 3). The curve at 25‰, 7 C: composite of data, Trial 5 and 6. For each curve: initial section — mean length of larvae samples throughout the hatching period; final section — posthatching growth in subsamples reared to yolk exhaustion and death from starvation. Arrows: MHT — mean hatching time; ML — calculated maximum length achieved.

TABLE 2. Incubation of flathead sole eggs, 1968 and 1969 trials. Total hatch refers to the percentage of eggs that hatched; viable hatch refers to the percentage of eggs producing larvae of normal appearance. For each trial the tabled values for number of eggs and percentage hatch represent averages from four subsamples.

Trial	Test conditions: Salinity (‰)	Test conditions: Temp (C)	No. of eggs per subsample (*mean*)	Total hatch (%)	Viable hatch (%)	Trial	Test conditions: Salinity (‰)	Test conditions: Temp (C)	No. of eggs per subsample (*mean*)	Total hatch (%)	Viable hatch (%)
	1968						*1969*				
1	15.00	3.00	191	43.66	6.66	1	13.00	4.00	114	58.10	33.38
2	15.00	9.00	155	97.72	77.64	2	13.00	10.00	124	59.38	28.27
3	35.00	3.00	127	32.74	24.51	3	37.00	4.00	129	60.40	47.95
4	35.00	9.00	179	86.77	82.99	4	37.00	10.00	126	74.44	69.69
5	25.00	6.00	179	87.57	70.35	5	25.00	7.00	129	88.90	74.92
6	25.00	6.00	153	98.09	94.88	6	25.00	7.00	104	96.40	92.02
7	12.85	6.00	166	96.19	79.23	7	10.42	7.00	131	80.80	53.58
8	37.15	6.00	158	91.69	89.18	8	39.58	7.00	139	85.90	72.65
9	25.00	2.36	152	18.26	9.39	9	25.00	3.36	132	58.59	50.64
10	25.00	9.65	157	93.96	91.75	10	25.00	10.65	127	82.82	78.60
11	20.00	4.50	167	65.81	36.39	11	19.00	5.50	109	82.09	79.13
12	20.00	7.50	180	92.30	90.00	12	19.00	8.50	114	88.94	64.04
13	30.00	4.50	127	91.60	89.51	13	31.00	5.50	126	46.91[b]	4.35[b]
14	30.00	7.50	189	38.88[a]	7.30[a]	14	31.00	8.50	139	84.09	81.82

[a,b]Initial inspection and preliminary computation indicated that these results most likely were anomalous. By means of a missing value technique they were replaced prior to final analysis with the following percentages of total and viable hatch, respectively: [a]88.95 and 79.80%; and [b]77.78 and 67.01%.

Both types of chromatophores became prominent on the walls of the hindgut and midgut. Yellowish-gold pigmentation also appeared on the dorsal surface of the brain, brain stem, and body wall dorsally, posteriorly as far as the hindgut. Melanophores also increased in numbers in the ventral finfold (Fig. 5d).

Decreasing spontaneous activity occurred in close association with exhaustion of yolk stores, followed by cessation of activity and death. Larvae reached maximum total length near the time yolk stores appeared to be exhausted in 50% of those measured, and subsequently decreased in length in most samples before death (Fig. 7). Maximum length attained in all trials varied between 6.3 and 7.2 mm. In those trials in which activity, feeding responses, and pigmentation appeared normal, and where mortality was low prior to yolk exhaustion, maximum size attained varied between 6.9 and 7.2 mm.

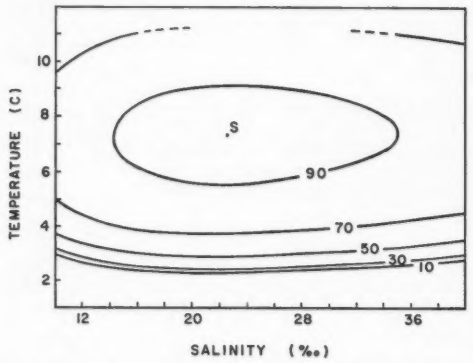

FIG. 8. Salinity–temperature response surface for flathead sole eggs showing calculated isopleths of percentage total hatch around a maximum at the center of the surface, S.

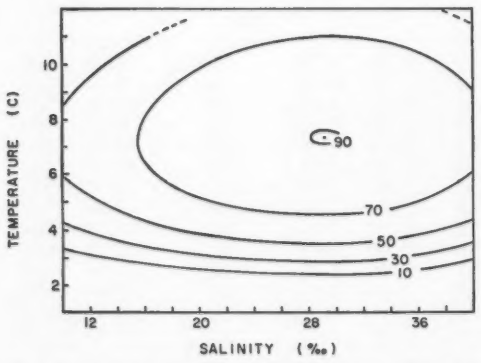

FIG. 9. Salinity–temperature response surface for percentage viable hatch of flathead sole eggs (see Fig. 8).

On the basis of the posthatching observations, normal development occurred mainly between 25 and 39.58‰, and at temperatures from near 5.5 to 10.65 C or greater. Considerable mortality occurred prior to yolk exhaustion at salinities below 20‰ (Table 3; Trial 7, 11, 12). In earlier, preliminary tests at 15‰ and 9 C, similar mortality occurred along with subintegumental swelling of the yolk sac, finfolds, and trunk posterior to the hindgut. Extensive mortality prior to yolk exhaustion also occurred among larvae reared at temperatures below 5.5 C (Table 3; Trial 3, 9). In preliminary tests (1968) larvae were reared to yolk absorption without difficulty at 30‰, 4.5 C. Early mortality in Trial 3 (37‰, 4.0 C) may represent osmoregulatory failure in a high salinity at a low temperature, or 4 C may be near the incipient low lethal temperature for flathead sole larvae. Early mortality in Trial 9 (25‰, 3.36 C) would appear to be a response to low temperature alone. In Trial 3 (37‰, 4.0 C) larvae emerging early in the hatching period were in a much less advanced state of development (see Fig. 5a) than those hatching either later in the same trial (see Fig. 5b) or in other trials. The two types of larvae from Trial 3 were reared in the usual manner. However, no differences could be detected in the sequence of developmental events and larvae in both subsamples achieved similar maximum total lengths prior to death. All of the observations suggest a low temperature limit for successful posthatching development is about 4.0–4.5 C.

Discussion

The pelagic nature of the flathead sole egg would subject it to a wide range of environmental conditions over the geographic range of the species. The hypothesis is proposed that salinity and temperature effects on egg and larval development, estimated from the experimental evidence, should provide some understanding of natural distribution and abundance of *H. elassodon*.

The laboratory observations will be considered with respect to 1) effects of salinity and temperature on development, and 2) other early life history studies on eggs and larvae of *Hippoglossoides*, and problems related to their identification. The available laboratory and field data will be compared and inferences drawn regarding distribution and abundance of the genus and, in particular, of *H. elassodon* in North American waters.

TABLE 3. Number of days from mean hatching time (MHT) to various events during posthatching development of unfed flathead sole larvae. Estimates in columns 3–9: based on observations at 2-day intervals; columns 10–11: calculated from distributions of mean length throughout and following hatching.

Trial	Rearing conditions: Salinity (‰)	Rearing conditions: Temp (C)	Larvae inactive	Active, feeding responses	Pigmentation bands, trunk and tail (*days*)	Decreasing activity	50% of larvae without yolk	Larvae moribund dying	Mortality complete	Maximum larval length attained after MHT: (*days*)	Maximum larval length attained after MHT: (*mm*)
10[a]	25.00	10.65	0–2	3	3	7–8	7–8	9–10	12	6.0	7.20
4	37.00	10.0	0–1	2–3	2–3	6–7	6–7	8–9	10	4.6	7.04
12	19.00	8.5	–	[b]	2–3	5–6	6–7	10–11	13	6.4	6.87
14	31.00	8.5	0–3	5–6	5–6	6–7	7	10–11	13	6.6	6.97
7	10.42	7.0	0–7	[c]	[c]	–	[c]	7	10	4.9	6.29
5,6	25.00	7.0	0–5	6–7	7–8	11–12	13–14	14	16	9.1–10.3	6.95–7.09
8	39.58	7.0	0–6	7–8	6–8	11–12	12–14	12	16	9.7	6.88
11	19.00	5.5	0–6	7–8	6–8	14	13–14	16[d]	22	11.6	6.85
3	37.00	4.0	0–7	8–9	9–10	[e]	15	8–9[e]	23	15.6	6.96
9	25.00	3.36	0–8	[f]	13	13	14	10–11	21	14.5	6.30

Trials (Tables 1,2; 1969 series) arranged in order of descending temperature. [b]Larvae inactive; dying before yolk utilized; yolk sacs distended, apparent osmoregulatory problems. [c]Larvae inactive; pale in appearance, pigmentation abnormal, yellow trunk bands present, melanophores present but contracted; yolk sacs distended, utilization abnormal; larvae dying before yolk exhaustion. [d]Two probable causes of mortality: larvae began dying on sixth day from apparent osmoregulatory problems; on sixteenth day from starvation. [e]Larvae relatively inactive; excessive mortality prior to yolk exhaustion considered an osmotic or a low temperature effect, or both. [f]Larvae relatively inactive; no feeding responses seen; excessive mortality prior to yolk exhaustion considered a low temperature effect.

Effects of Salinity and Temperature on Development

Ninety percent total hatch is predicted over the range of 14–35‰ (Fig. 8). Maximum viable hatch is predicted to occur at or near 29.16‰ (Fig. 9) within the plausible range of 28–32‰. Fifty percent viable hatch is predicted to occur over the entire salinity range investigated (10.42–39.58‰). Therefore, incubating eggs of *H. elassodon* in southern British Columbia waters are considered euryhaline.

After hatching, however, larvae appear more susceptible to salinity extremes (Table 3). The lower boundary of physiologically compatible salinities for normal larval development appears to be near 20‰, while the upper boundary would not be encountered under natural conditions. Optimum salinities for larval development are taken to be those associated with maximum viable hatch, namely 28–32‰.

The current study indicates that buoyancy changes in developing eggs may be complex; both egg diameter and egg density appear interdependent, and both appear to respond to changes either of salinity or temperature. Catch records and oceanographic data (Pertseva-Ostroumova 1961; Dodimead et al. 1963; Natarov 1963; Plakhotnik 1964; Shurunov 1964) indicate that *H. elassodon* would be found in regions with salinities of 27–34‰. Egg density measurements suggest that eggs of *H. elassodon* would be found in salinities greater than 19–20‰; that is, eggs would rise into the surface layers of 27‰ or greater, or sink from freshened surface layers to deeper, more saline waters of 19–20‰ or greater. The results suggest that egg density may assist in the maintenance of eggs and larvae of *H. elassodon* at physiologically compatible salinities of 20‰ or greater.

Effects of temperature on egg development and survival are more narrowly defined by the experimental results. Total hatch and viable hatch were both maximized at temperatures at or near 7.3–7.4 C within a plausible range of 7.0–7.5 C. Within the salinity range of 20–34‰, estimated in the previous section, the boundaries of 50% viable hatch are predicted at temperatures of 3.5–4.0 C and >10.65 C (Fig. 9). Maximum larval size at hatching is poorly defined by the temperature data, but probably occurs between 6 and 10 C.

While egg development may be limited at temperatures of 3.5–4.0 C, larval survival appears to require marginally higher temperatures of 4.0–4.5 C. Above this level larvae developed normally, without undue mortality prior to yolk exhaustion, up to and including the highest temperatures tested (10.0–10.65 C; Table 3). However, assuming that maximum size at hatching and maximum growth rate after hatching have survival value, then temperatures of 6–10 C are concluded to favor continued larval development. Those salinity–temperature conditions estimated to favor or limit development and survival of eggs and larvae in the current study are summarized in the following table.

	Salinity (‰)			Temp (C)		
Stage	Lower limit	Optimum	Upper limit	Lower limit	Optimum	Upper limit
Egg	14–15	29.16	35–37	3.5–4.0	7.3–7.4	>10.65
Larva	~20	28–32	35–37	4.0–4.5	6–10	>10.65

Problems of Identification, Early Life History Stages

Further examination of the hypothesis relating salinity and temperature effects to development of eggs and larvae of *H. elassodon* becomes complex. As noted earlier, adults of the three forms of *Hippoglossoides* cannot be separated with certainty on the basis of meristic characters. Early life history studies have employed egg and larval characteristics to distinguish between the three forms in Asian waters. However, as will be shown by comparison of available data and the current laboratory information, the characteristics used may not provide an unequivocal separation of the three forms.

Eggs — Differences in total diameter, yolk diameter, and perivitelline space have been used as criteria for identifying eggs of *H. elassodon*, *H. robustus*, and *H. dubius*. Most eggs examined have been obtained from ichthyoplankton samples in Asian waters. From such material it has been concluded that total diameter and yolk diameter are greatest in *H. elassodon*, intermediate in *H. robustus*, and smallest in *H. dubius* (Pertseva-Ostroumova 1961). For the three forms, marked variations in egg measurements occur in samples of fertilized, unfertilized, live, and preserved eggs (Table 4).

A comparison of the available data suggests that fertilized, preserved eggs of 1) *H. elassodon* in Asian and North American waters are within the same size range; 2) *H. robustus* and *H. dubius* are very similar in size and possibly are smaller than those of *H. elassodon*. However, the suggested differences in size among the three forms are small, relative to the variability occurring between samples. Moreover, the mean value for total diameter of live, fertilized eggs of *H. dubius* from Ussuri Bay (Table 4; 2.31 mm) appears to have been obtained before the eggs reached maximum total diameter (Pertseva-Ostroumova 1961; her table 7). The sources of variability documented in the current study can produce a 10–20% variation in egg diameter. When this potential error is related to the size ranges of

fertilized, preserved eggs of the three species, the ranges could overlap considerably. In summary, North American and Asian *H. elassodon* appear indistinguishable on the basis of egg size. Although the eggs of *H. robustus* and *H. dubius* appear somewhat smaller than those of *H. elassodon*, drawing of that distinction should await further confirming evidence.

If laboratory or field collections are employed to distinguish between species of *Hippoglossoides*, sources of variation in egg size should be examined further. These would include 1) the time course of water intake and swelling of newly fertilized eggs; 2) differences between females; 3) differences associated with salinity and temperature conditions of incubation, and possibly of fertilization; 4) differences arising from methods of preservation; and 5) changes occurring as a result of storage (see Thompson and Van Cleve 1936; their table 9).

Larvae — Hippoglossoides elassodon and *H. dubius* are separated from larvae of other North Pacific genera by the presence of four tailbands of chromatophores in older larvae (Pertseva-Ostroumova 1961). The larvae of *H. robustus* are as yet poorly known, although pigmentation patterns of older juveniles suggest that the larvae also possess the four tailbands. *H. elassodon* is distinguished by the presence of scattered melanophores in the finfold surrounding the tip of the tail (Fig. 5), a characteristic not found in *H. dubius*. *H. dubius* also has been separated on the basis of small size of larvae (3.5 mm or larger)

TABLE 4. Compilation of size measurements for fertilized, unfertilized, live, and preserved[g] eggs of North Pacific Ocean *Hippoglossoides*.

Species and location	Total diameter (TD) mean (*mm*)	Total diameter (TD) range (*mm*)	Yolk diameter (YD) mean (*mm*)	Yolk diameter (YD) range (*mm*)	Perivitelline space (PVS) (*mm*)	PVS/TD (%)
H. elassodon — unfertilized, live						
Puget Sound, Wash., USA[a]	–	1.3–1.5	–	–	–	–
Strait of Georgia, B.C.[b] — unfertilized, preserved	1.31	–	–	–	–	–
Puget Sound, Wash.[a,g] — fertilized, live	1.4	1.3–1.5	–	–	–	–
Puget Sound, Wash.[a]	–	2.3–2.4	–	–	0.9–1.1	39–46
Puget Sound, Wash.[a]	2.4	2.1–2.7	–	–	0.8–1.3	42
Strait of Georgia, B.C.[b] — fertilized, preserved	2.48	1.97–2.96	1.52	1.47–1.55	–	25–48
Puget Sound, Wash.[a]	2.5	2.1–3.2	–	–	1.0–(1.4)[i]–2.2	57
Gulf of Alaska[c,g]	3.27	2.75–3.75	–	–	–	–
"Soviet waters"[d]	3.02–3.26	2.45–3.67	1.22–1.33	1.10–1.53	–	29–(31)–33
SW coast, Kamchatka Peninsula[e]	–	2.21–3.28	–	–	–	–
Central and SE Bering Sea[f,h]	–	2.4–3.55	–	–	–	–
H. robustus — fertilized, preserved						
SW Bering Sea[d]	2.27–2.54	2.04–2.69	0.98–1.10	0.86–1.14	–	26–(29.5)–32
Chukchi Sea[d]	–	2.17–2.46	–	0.90–1.02	–	25
H. dubius — unfertilized, live						
Ussuri Bay, USSR[d]	1.23	1.17–1.28	–	–	–	–
H. dubius — fertilized, live						
Ussuri Bay[d]	2.31	2.02–2.42	–	–	–	20–(23.7)–25.2
Ussuri Bay[d] — fertilized, preserved	2.27	2.02–2.34	1.03	0.97–1.1	–	–
Ussuri Bay[d]	–	–	–	–	–	32
Peter the Great Bay, USSR[g,h]	2.37–2.62	2.02–2.94	0.90–0.92	0.82–1.02	–	20–(27–30)–33

[a]Miller (1969); 48°40′N, 123°54′W. [b]This paper. [c]Thompson and Van Cleve (1936). [d]Pertseva-Ostroumova (1961). [e]Rass and Zheltenkova (1948); 53°00′N, 156°00′E. [f]Musienko (1963). [g]Preservatives: Miller — Stockard's solution (5% formalin-4% glacial acetic acid, 6% glycerin, 85% sea water); Thompson and Van Cleve — 3–4% formaldehyde; Pertseva, Ostroumova — 2% formalin. [h]Interpreted as preserved material. [i]Means in parentheses.

and the presence of evenly distributed melanophores in early larvae. Some of the more specific larval characteristics are summarized in Table 5.

From the tabled data no obvious size differences between North American and Asian *H. elassodon* are detectable (Table 5). There may be differences in the time of first appearance of the melanophore tailbands, and in the location of the anterior tailband relative to the hindgut. The band pattern in Asian *H. elassodon* is said to develop prior to hatching. In the current study, band formation occurred after hatching (Table 3).

In general, differences between larvae of the three forms of *Hippoglossoides* are difficult to evaluate, particularly when the criteria employed are temperature dependent. Some of the differences (Table 5) may be a function of environmental conditions during fertilization or incubation. In her original data on laboratory incubation of *H. dubius*, Pertseva-Ostroumova (1961) states incubation time to be the period between fertilization and first hatch. Compared on that basis, the time from fertilization to appearance of first hatched larvae in the current study (9.65–10.65 C) agrees closely with Pertseva-Ostroumova's incubation times to first hatch; rate of development of *H. dubius* (fertilization to estimated mean hatching time) follows the same pattern of developmental velocity as that of *H. elassodon* (Fig. 4).

Size of *H. dubius* larvae obtained from Pertseva-Ostroumova's laboratory incubations may now be compared with that of *H. elassodon* incubated at comparable temperatures in the current trials. However, again Pertseva-Ostroumova evidently measured those larvae hatching in the first stage of the hatching interval. As *H. elassodon* larvae hatch at progressively larger sizes throughout the hatching interval (Fig. 7), the comparison in the following table is made on the basis of mean total length of live larvae for the first 16 h of the hatching interval:

Species	Total length	
	Mean (*mm*)	Range (*mm*)
H. dubius; reared at 10.7, 10.8 and 12.1 C	3.30	3.00–3.40
H. elassodon; reared at		
1968: 25‰, 9.65 C	4.08	3.52–4.43
1969: 13‰, 10.0 C	3.72	3.22–4.11
37‰, 10.0 C	2.94	2.25–4.43
25‰, 10.65 C	2.91	2.58–3.94

The comparison points up the necessity of considering not only temperature experience during incubation, but also those changes in size of newly hatched larvae associated with time of appearance in the hatching interval. On this basis it appears impossible to detect any real differences in total length of *H. elassodon* and *H. dubius* larvae emerging in the first part of the hatching interval. Furthermore, no conclusive differences are indicated in total lengths of older larvae (Table 5). It is concluded that separation of North American *H. elassodon* and *H. dubius*, based on size differences of their larvae, must await further confirmatory evidence. As North American and Asian *H. elassodon* larvae were found to be similar in size, all three forms appear indistinguishable on the basis of total length at comparable developmental stages.

Remaining characteristics (Table 5) used in separating the three species of *Hippoglossoides* involve the location and density of larval melanophore accumulations. Notable is the observation of presumptive tailband accumulations prior to hatching in Asian *H. elassodon*; these do not appear in North American *H. elassodon* larvae until several days after hatching and their rate of appearance is temperature dependent (Table 3). In addition, the density of finfold melanophore pigmentation may separate *H. dubius* and *H. robustus* from either North American or Asian *H. elassodon*. However, a quantitative documentation of pigmentation patterns has not been carried out among the three forms. Until the extent of variability in melanophore pattern location and density is defined relative to larval size, developmental age, and conditions of incubation, the validity of using such differences as a means of species separation remains open to question.

Distribution and Abundance of *H. elassodon*

Relative abundance of *H. elassodon* in different geographic regions is not well defined. Although *H. elassodon* is the only recognized member of the genus south of the Aleutian Islands, its identification and distribution in other areas of the North Pacific are less certain.

Most catch records in the Bering Sea have not distinguished between *H. elassodon* and *H. robustus*, while over the estimated range of *H. elassodon* few quantitative catch records are available. Yet, in order to relate current experimental findings to hydrographic conditions over the range of *H. elassodon*, some further consideration must be given the known distribution and abundance of *Hippoglossoides* in North American and Asian waters.

Geographic range — In North American trawl catches, *H. elassodon* is uncommon or incidental

TABLE 5. Comparison of larval characteristics of *Hippoglossoides*. The bulk of the table is extracted from Pertseva-Ostroumova (1961) and comparisons are made with North American larvae. Means of measurements are shown in parentheses.

Characteristic		North American *H. elassodon*	Asian *H. elassodon*	*H. robustus*	*H. dubius*
No. of times larvae encircle yolk prior to hatching		3/4[a]	1–1/2	–	3/4
Size of larvae (*mm*)					
at hatching	(live)	3.23–6.27[c,f]	–	–	3.0–(3.3)–3.4
	(fixed)	4.5–(4.6)–4.7[b]	5.3–(6.4)–6.94[g]	–	2.60–(3.0)–3.13
at yolk absorption	(live)	6.29–7.20[e] 6.60–(6.9)–7.20[b]	5.0–6.64[h]	–	5.28–5.75
	(fixed)	5.2–(5.6)–6.0[b]	5.39–(6.15)–6.97[g]	–	5.10–(5.40)–5.60
after yolk absorption	(fixed)	4.8–(5.2)–5.8[b]	6.10–(6.58)–7.12[i]	–	4.97–(5.20)–5.40
Yolk-sac length at hatching	(live)	1.16–1.91[d]	–	–	–
	(fixed)	–	0.79–1.01[g]	–	1.15–(1.26)–1.38
Pigmentation of embryo begins		prior to completion of tail-bud formation	prior to completion of tail-bud formation	prior to completion of tail-bud formation	prior to completion of tail-bud formation
Melanophore bands					
number		4	4	4	4
first occurrence		posthatching (2–13 days at 3.4–9.65 C)	prehatching	–	posthatching (3+ days at 13 C)
location		accumulation of melanophores above hindgut; origin of first band variable, starting on 2nd–5th myotome posterior to hindgut[a]	accumulation of melanophores above hindgut; origin of first band posterior to hindgut on tail myotomes 7–9[j]	–	accumulation of melanophores above hindgut, origin of first band posterior to hindgut on tail myotomes 5–10
Melanophores on finfold surrounding					
tip of tail		present	present	absent	absent
on dorsal margin of tail finfold		usually present	usually present	usually absent	usually absent

[a]This paper. [b]Miller (1969). [c]At mean hatching time, various temperatures (this paper). [d]From Fig. 4. [e]Calculated maximum lengths attained (this paper) [f]Miller (1969) obtained larvae at 9.5 C: 5.5–(6.0)–6.3 mm. [g]Field collection; material may have been somewhat older than stated. [h]"Recently hatched." [i]Range also shown as 6.10–7.42 in original text. [j]Estimated from sketches in original reference.

from its most southerly recorded point of capture (Point Reyes, California) to Cape Spencer, Alaska. Considerable numbers have been taken in the Gulf of Alaska–Alaska Peninsula region (Alverson et al. 1964) (Table 6). No distinction has been made in the Bering Sea between *H. elassodon* and *H. robustus* in commercial fishing operations, although the two species comprise about 5% by weight of flounder catches in the eastern Bering Sea (Table 6) (Forrester et al. 1974). Okada and Kobayashi (1968) report both species from that region, but no estimate is available on the proportion of *H. elassodon* in the eastern Bering Sea population. Tentatively, it is concluded that *H. elassodon* occupies the eastern Bering Sea flats and extends along the edge of the flats as far as the southern reaches of Anadyr Gulf, USSR. From Anadyr Gulf its distribution continues southwestward along the coast around Kamchatka Peninsula to about 56°N lat. in the Sea of Okhotsk.

Hippoglossoides robustus occupies the same range as *H. elassodon* from the eastern Bering Sea to the northern shores of the Okhotsk Sea. However, *H. robustus* extends into the Chukchi Sea, generally throughout the Okhotsk Sea, and as far south as the southern shores of Sakhalin, Aniva Gulf, and the northern shores of Hokkaido.

Hippoglossoides dubius is centered in the Sea of Japan from the southeast coast of Korea to the northern part of Tartar Strait. In Japanese waters it extends from Shimane Prefecture on the western shores of Honshu (35°30 N, 133°E) (Okada 1966), around northern Hokkaido, and as far south as Tokyo Bay (Moiseev 1953) on the east side of Honshu. (Although the species is listed by Okada as *H. elassodon*, the southern part of the described distribution appears to correspond with that of *H. dubius*.)

Hydrography — Depth distribution of the three species of *Hippoglossoides* ranges from a few meters to depths of 350 m or more. In Asian waters the genus is generally caught in greatest numbers at mean depths of 75–150 m. In North American waters south of the Bering Sea substantial catches are made at depths of 91–364 m (Table 6). In Asian and Bering Sea waters *Hippoglossoides* performs marked bathymetric migrations relative to season and state of sexual maturity. There, *Hippoglossoides* migrates to deeper, warmer waters of the outer continental shelf and upper slope with the approach of winter. With warming of the upper mixed layer in hydrological spring, an inshore movement occurs to shallower waters (50–100 m). There is a dispersal after spawning into both shallow-

TABLE 6. Distribution of *H. elassodon* in North American waters, summarized from Alverson et al. 1964.

Occurrence	Region[a]				
	Oregon–Washington	B.C.–SE Alaska	Gulf of Alaska	Alaska Peninsula	Bering Sea
% by weight of total flounders	Trace	0.4	6.5	8.1	5.4[b]
% of species occurrence	1.9	5.5	48.0	67.6	–
% of total catch	T	0.1	3.4	4.5	4.4[b]
Depth of maximum abundance (*m*)	91–364	274–364	91–181	182–273	2–90
lb/h of trawling by depth intervals (*m*)					
2–90	0	0	16	21	97[b]
91–181	T	1	83	80	–
182–273	T	1	67	145	–
274–364	T	10	62	31	–
365–547	0	–	0	2	–

[a]Oregon–Washington — California border to Juan de Fuca Canyon; B.C.–SE Alaska — Juan de Fuca Canyon to Cape Spencer, Alaska; Gulf of Alaska — Cape Spencer to west end of Kodiak Island; Alaska Peninsula — west end of Kodiak Island to Unimak Pass; Bering Sea — South and east of a Pribilov–Nunivak Island transect. [b]Mixture of *H. elassodon* and *H. robustus*.

er and deeper waters, coincident with a period of intense feeding activity (Moiseev 1953).

In the southeastern Bering Sea, *H. elassodon* winters on the slope and lower part of the continental shelf at depths from 70 to 400 m (Fadeev 1965) where bottom temperatures are about 2.5 C on the outer shelf and 3.5–4.0 C on the slope (Natarov 1963). Bottom temperatures there remain in the 2–4 C range in summer; however, marked warming of the upper layers occurs with temperatures reaching 4–10 C above 50–75 m.

In the Gulf of Alaska over the upper continental slope and shelf, temperatures in the upper zone vary between 3.0 and 3.5 C and 4.5 and 5.0 C in winter and from 5 C to 10–13 C in summer (Dodimead et al. 1963). Temperatures in the stable, intermediate layer (75–350 m) reflect properties of the northward-moving subarctic current. There exists a subsurface thermal maximum, affecting bottom temperatures over much of the upper slope and shelf waters in the Alaska Peninsula–Gulf of Alaska region. Under the influence of this thermal maximum in the intermediate layer, bottom temperatures range in general from 4.0 to 5.5 C (250–300 m) in Alaska Peninsula waters, and from 4.0 to 6.0 C (140–350 m) in the Gulf of Alaska (Plakhotnik 1964; Shurunov 1964).

In North American waters south of the Bering Sea, evidence relating distribution and movements of *H. elassodon* to depth and temperature is rather meager. In the Gulf of Alaska, maximum catches are made in the 91–273-m depth interval (Table 6). Experimental trawl catches of Westrheim and coworkers (Westrheim 1967; Levings 1968; Westrheim et al. 1968; Harling et al. 1968, 1970) conducted in waters of 90 m and deeper found some concentrations of *H. elassodon* in depths of 120–350 m at temperatures of 4.3–5.8 C during January–June, and in 90–260 m at temperatures of 4.8–7.8 C during July–September.

Another series of experimental trawls (Best 1969a, b; International Pacific Halibut Commission unpublished data) conducted in this region sampled predominately the shallower waters; the data suggest that a further, significant component of the population may be found within the 0–90-m depth interval in the summer period. Temperatures and depths occupied suggest that *H. elassodon* is associated with the region of the upper slope and shelf influenced by the subsurface thermal maximum.

It is notable that most experimental hauls reported by Hitz and Rathjen (1965) in the Gulf of Alaska west of about 144°W long (Kayak Island) contained *H. elassodon*. East of Kayak Island many successful hauls did not. Temperature of the thermal nucleus in the stable intermediate layer east of Kayak Island is about 6.2 C or greater; west of Kayak Island it decreases from 6.2 C to 5.0 C south of Unimak Island (Plakhotnik 1964).

South of Cape Spencer abundance of *H. elassodon* drops substantially. Associated with this change in abundance is the northern boundary of the Canadian water mass (Kostikova 1965). This water mass, which approximates the upper transitional zone of Dodimead et al. (1963), is characterized by temperatures of 6 C or greater at depths of 200 m or less; in general it influences bottom temperatures at its northern extent to a point between the British Columbia–Alaska boundary and Cape Spencer. Associated with the northern region of the Canadian water mass and the abrupt decline in abundance of *H. elassodon* southward is the location of the 6.0–6.5 C isotherms. These isotherms are associated with the halocline at depths of 50–100 to 150–200 m.

In British Columbia waters *H. elassodon* becomes an incidental species. In southern British Columbia waters, water temperatures at the bottom of the upper zone (75–150 m) increase to about 8 C (7–10 C), and surface temperatures in and after the spawning period (April–May) range from 8 to 11 C (Hollister and Sandnes 1972). South of this region *H. elassodon* becomes uncommon.

Experimental fishing in British Columbia waters (Westrheim and co-workers) in depths greater than 90 m found limited quantities of *H. elassodon* in depths of 90–210 m at temperatures of 6.0–8.3 C. In water shallower than 90 m the species is not abundant enough to attract commercial fishing.

Available information relating temperature and depth to biological events is summarized in Table 7 for both North American and Asian *H. elassodon*, and for *H. robustus* and *H. dubius*. In general the three Asian forms appear more cold-tolerant than the North American form of *H. elassodon*. However, any real distinction between temperature characteristics of the three or possibly four forms of *Hippoglossoides* must await an examination of their temperature tolerances under controlled conditions.

Ecological Implications

Developmental characteristics were determined for eggs and larvae of *H. elassodon* obtained from a spawning population in British Columbia waters. This population was near the southern boundary of the range where *H. elassodon* is an incidental species. The experimental findings may more properly refer to characteristics of a southern subpopulation of North American *H. elassodon*. Nevertheless, over the North American range of the species, the characteristics considered appear to relate to one population. In terms of the experimental and ecological information reviewed, the

following hypothesis is advanced regarding distribution and abundance of North American *H. elassodon*.

1. The center of abundance of the North American population east and south of the Alaska peninsula appears to be from Unimak Island to Kayak Island in the Gulf of Alaska. The range extends northward where substantial numbers, in association with *H. robustus*, probably are limited to the southeastern Bering Sea in the summer period. The population probably is contiguous with Asian *H. elassodon* through an attenuated link along the outer edge of the Bering Sea flats. In the Gulf of Alaska the population appears to diminish rapidly in the region of Cape Spencer, southeastern Alaska. In British Columbia waters, *H. elassodon* becomes an incidental species; south of British Columbia it becomes uncommon.

2. In the presumed center of population abundance in the Gulf of Alaska, bottom temperatures range from about 4.0 to 6.0 C in the presumed spawning season. In the eastern Bering Sea, populations appear linked to the availability of bottom temperatures of 2.5–3.0 C or higher in the spawning period. The location of the 6.0–6.5 C isotherms in the halocline in the vicinity of Cape Spencer, southeastern Alaska, appears associated with the marked reduction in population southward. In British Columbia waters, where *H. elassodon* is

TABLE 7. Average catch of *H. elassodon* (lb/h) in relation to bottom temperature and depth for a series of experimental trawls in the Gulf of Alaska–Alaska Peninsula, and in British Columbia waters. (Number of trawls shown in parentheses.)[a]

Gulf of Alaska and Alaska Peninsula

	Depth interval of drag (m)								
	0–44	45–90	91–135	136–181	182–227	228–273	274–318	319–364	>364
Jan.–Mar.	–	–	120(1)	4(1)	52(8)	40(1)	10(3)	9(3)	0(2)
Apr.–June	–	–	–	–	81(3)	0(7)	0(4)	0(2)	0(2)
July–Sept.	–	4(6)	29(34)	108(42)	14(34)	92(23)	1(4)	0(1)	0(2)

	Bottom temperature (C)								
	3.5–3.99	4.0–4.49	4.5–4.99	5.0–5.49	5.5–5.99	6.0–6.49	6.5–6.99	7.0–7.49	7.5–7.99
Jan.–Mar.	0(1)	5(2)	49(7)	56(5)	3(2)	0(2)	–	–	–
Apr.–June	–	0(7)	0(2)	0(4)	48(5)	–	–	–	–
July–Sept.	–	35(3)	21(11)	48(26)	41(47)	28(6)	144(27)	1(5)	1(1)

British Columbia waters

	Depth interval of drag (m)								
	0–44	45–90	91–135	136–181	182–227	228–273	274–318	319–364	>364
Jan.–Mar.	6(3)	<1(13)	15(43)	6(13)	0(5)	0(1)	0(5)	119(3)[b]	0(2)
Apr.–June	0(1)	0(1)	8(2)	0(1)	0(1)	0(2)	0(1)	0(2)	–
July–Sept.	0(1)	–	23(6)	14(5)	2(11)	0(6)	0(5)	–	–

	Bottom temperature (C)									
	3.5–3.99	4.0–4.49	4.5–4.99	5.0–5.49	5.5–5.99	6.0–6.49	6.5–6.99	7.0–7.49	7.5–7.99	8.0–8.49	8.5–8.99
Jan.–Mar.	0(1)	0(1)	–	1(1)	0(11)	2(24)	0(8)	1(18)	67(11)[b]	31(9)	0(4)
Apr.–June	–	0(1)	0(2)	0(1)	0(1)	–	–	0(1)	8(2)	0(3)	–
July–Sept.	–	–	-	–	0(1)	2(19)	6(8)	17(3)	44(2)	0(1)	–

[a]From records of Westrheim and co-workers; see text. [b]The figure includes one substantial catch in Rivers Inlet (Westhreim 1967).

an incidental species, bottom temperatures in the spawning season range from 6 to 8 C.

3. During and following the spawning season, temperatures to which eggs would be exposed during incubation range from 4 to 6 C in bottom waters with limits of about 3 and 8 C. Surface temperatures range from about 4 to 10 C in the southeast Bering Sea, 7–11 C in the Gulf of Alaska–Alaska Peninsula region, and 8–11 C in British Columbia waters. Salinities in the water column generally would be 27‰ or greater. Salinity of neutral buoyancy of North American *H. elassodon* eggs is assumed to range from near 25‰ in early stages to 18–20‰ in prehatching eggs and newly hatched larvae. Therefore, eggs and larvae would be expected to concentrate in surface or near-surface waters where temperatures are consistent with predicted maximum (7.2–7.3 C) or high (6–10 C) survival. It is presumed that survival at the northern end of the range would be marginal for eggs developing at 3.5–4.0 C and larvae developing at 4.0–4.5 C. At the southern end of the range it is assumed that spawning may be inhibited at bottom temperatures greater than 8–9 C, even though egg and larval survival would be reasonable at temperatures of 10.65 C or several degrees Celsius higher.

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Observations on Subsurface Distributions of Chlorophyll *a* and Phytoplankton Carbon in the Northeast Pacific Ocean

LOUIS A. HOBSON AND DAWN E. KETCHAM[1]

Department of Biology
University of Victoria, Victoria, B.C. V8W 2Y2

HOBSON, L. A., AND D. E. KETCHAM. 1974. Observations on subsurface distributions of chlorophyll *a* and phytoplankton carbon in the northeast Pacific Ocean. J. Fish. Res. Board Can. 31: 1919–1925.

Measurements of subsurface concentrations of chlorophyll *a* and phytoplankton carbon were made between Vancouver Island and Hawaii during summer 1971. In general, chlorophyll *a* concentrations increased and concentrations of phytoplankton carbon decreased from 10 m to depths varying between 100 and 150 m. These distributions indicated that cellular contents of chlorophyll *a* increased as cells were exposed to diminishing daily irradiations. Daily compensation irradiations for net photosynthesis of subsurface phytoplankton in the northeast Pacific Ocean were estimated in summer 1973. Values ranged between 0.5 and 0.7 cal cm^{-2} but variability among replicates was large giving a range of 0 and 1.2 cal cm^{-2}. It was not possible to correlate compensation irradiations with taxonomic composition of phytoplankton populations.

HOBSON, L. A., AND D. E. KETCHAM. 1974. Observations on subsurface distributions of chlorophyll *a* and phytoplankton carbon in the northeast Pacific Ocean. J. Fish. Res. Board Can. 31: 1919–1925.

Nous avons mesuré les concentrations de chlorophylle *a* et de carbone phytoplanctonique sous la surface entre l'île Vancouver et Hawaii au cours de l'été 1971. En général, la concentration de chlorophylle *a* augmente et celle du carbone phytoplanctonique diminue à partir de 10 m jusqu'à une profondeur de 100 à 150 m. Ces distributions indiquent que le contenu cellulaire en chlorophylle *a* augmente quand les cellules sont exposées à des irradiations journalières décroissantes. Durant l'été 1973, nous avons estimé l'irradiation de compensation journalière pour la photosynthèse nette du phytoplancton sous la surface, dans le nord-est de l'océan Pacifique. Les valeurs vont de 0.5 à 0.7 cal cm^{-2}, mais les répliques accusent de grandes variations dans un intervalle de 0 à 1.2 cal cm^{-2}. Il nous a été impossible d'établir une corrélation entre les irradiations de compensation et la composition taxonomique du phytoplancton.

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SUBSURFACE chlorophyll maxima are recognized as transitory features of large scale occurring in tropical and temperate seas (e.g. Anderson 1969; Hobson and Lorenzen 1972; Kawarada and Sano 1969; Nishizawa et al. 1971; Takahashi et al. 1972). Possible mechanisms producing chlorophyll maxima are accumulation of chlorophyll *a* in individual phytoplanktonic cells existing at depths near the bottom of the euphotic zone (Steele 1964), and accumulation of cells at the bottom of the euphotic zone (Steele and Yentsch 1960). Recently, information that suggests that subsurface chlorophyll maxima in sea water and fresh water are due to this latter mechanism has been accumulating (Hobson and Lorenzen 1972; Kiefer et al. 1972; Taniguchi and Kawamura 1972). However, these observations have been confined to small geographic areas.

Anderson (1969) and Hobson and Lorenzen (1972) noted that large-scale distributions of subsurface maxima were patchy. The latter authors suggested that this patchiness might be a function of the taxonomic composition of phytoplankton making up maxima because different taxonomic groups might display different compensation irradiations.

Purposes of the present research were 1) to obtain information about subsurface distributions of

[1]Present address (D.E.K.): Harmac Pulp Limited, Nanaimo, B.C.

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chlorophyll *a* and phytoplankton biomasses and taxonomic compositions in a large area of the northeast Pacific Ocean during summer; and 2) to determine compensation irradiations and taxonomic compositions of cells collected from subsurface populations.

Materials and Methods

Stations were occupied by CNAV *Endeavour* along a track from Vancouver Island to Hilo, Hawaii during August 1971, and by RV *T.G. Thompson* at stations bounded by 41° N and 50° N lat. and by 135° W and 145° W long. during July and August 1973 (Fig. 1). Water samples were collected at various depths between the sea surface and 200 m by 4-liter plastic sample bottles at various times each day.

The distribution of density was determined from temperature and salinity data. Temperature and salinity were determined by reversing thermometers and by conductimetric techniques, respectively. Concentrations of nitrate and nitrite ions were determined by a technique outlined in Strickland and Parsons (1968), and concentrations of chlorophyll *a* were measured and were corrected for interference by other pigments with the acetone-extraction technique and Turner Model 111 fluorometer described by Lorenzen (1966). Irradiance by wavelengths from 0.3 to 3 μm at the sea surface was measured by a Star Pyranometer connected to a strip-chart recorder. These measurements were reduced by 50% to give photosynthetically active irradiance, and these latter values were reduced by 5% to give an approximate correction for reflection of light from the sea surface based on information presented by Jerlov (1968). Attenuation coefficients of the sea water were calculated with the equation:

$$K = 1.7/D,$$

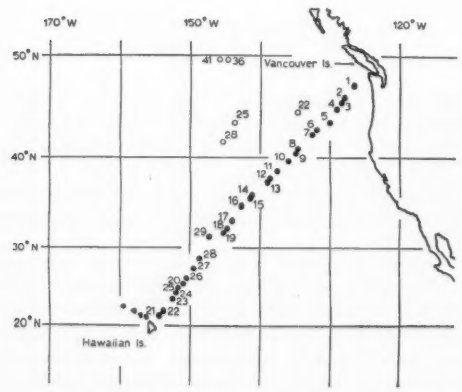

FIG. 1. Stations occupied by CNAV *Endeavour* (●) during August 1971 and by RV *T.G. Thompson* (○) during July–August 1973.

where K is the extinction coefficient (m^{-1}) and D is the maximum depth (meters) at which a Secchi disk can be seen. These coefficients are minimal estimates because turbidity of water must increase if concentrations of subsurface phytoplankton increase below a depth of about 30 m, and these increases in turbidity are not measured by the Secchi disk. This source of systematic error is expected to be maximal at inshore stations where concentrations of subsurface phytoplankton may be larger than concentrations at offshore stations. Phytoplankton was preserved with Lugol's solution (acetate–iodine), and concentrations of phytoplankton carbon were determined with the technique described by Hobson et al. (1973). This technique includes counting cells with an inverted microscope, measuring dimensions of cells with an ocular micrometer, computing cell volumes with formulae of volumes of geometrical solids, and converting cell volumes to cell carbon by conversion factors selected from the literature.

Responses of photosynthetic rates of phytoplankton collected either from subsurface chlorophyll maxima or from 1% light depths to variations in irradiation were determined by the ^{14}C technique, ignoring losses of ^{14}C contained in excreted organic compounds. Seawater samples were transferred in darkened containers to a controlled environment chamber on shipboard. Then, 20 μCi of $H^{14}CO_3^-$ were added to a 1-liter sample, and this sample was dispensed into 12 clear and 4 dark, 30-ml culture tubes. Three clear tubes and 1 dark tube were placed in a box with five black sides and one clear side to give illumination of the long axis of the tubes by cool-white fluorescent lights. A neutral density filter (cheese cloth) and a color filter (Cinemoid #42 pale violet) were attached to the clear side of the box to regulate the quantity and the quality of light within the box. For each sample, four boxes were set up to give four different irradiances varying from 0.5 to 9.4% of sea surface irradiance. Illumination in the boxes was measured by a Weston Model 756 Sunlight Illumination Metre equipped with a quartz filter. Illuminance was converted to irradiance using the spectral response of Weston Selenium Barrier Layer cells with quartz filters and the wavelength of maximum transmission of the colored filter (440 nm). Lights were turned on ½ h after local sunrise and were turned off ½ h before local sunset. Temperature of the controlled environment chamber was set to correspond approximately to the temperature at the depth from which samples were collected. Duration of experiments was 24 h, after which samples were filtered through HA Millipore filters (0.45 μm pore diameter). Then, filters were rinsed with filtered sea water and were stored frozen until fumed with HCl for 10 min. They then were dried at 35 C for 20 min and were placed in 10 ml of Econofluor (New England Nuclear). Carbon-14 activity was measured by a Beckman LS-133 liquid scintillation counter. Activity in dark tubes was subtracted from activity in light tubes because dark-tube activity was identical to background activity in $H^{14}CO_3^-$ solutions (New England Nuclear), presumably due to contamination of solutions by particles containing ^{14}C.

Results

Stormy weather was experienced between stations 1 and 4 of the Hawaii cruise and sea surface irradiation increased from 160 cal cm^{-2} day^{-1} to 270 cal cm^{-2} day^{-1} from station 1 to station 4. Seaward of station 4, variation in sea surface irradiation was small, averaging 300 cal cm^{-2} day^{-1}. During the *T. G. Thompson* cruise, sea surface irradiation varied between 121 and 262 cal cm^{-2} day^{-1}.

A chlorophyll maximum occurred between depths of 40 and 75 m from station 1 (280 km offshore Cape Disappointment, Oreg., USA) to station 7 (550 km offshore Cape Blanco, Calif., USA). The maximum concentration of this pigment occurred at station 3 and it was centered at 50 m (Fig. 2). This depth was at the 1% light depth (Fig. 2) and was below the pycnocline (Fig. 3) where concentrations of NO_3-N and NO_2-N were increasing from undetectable levels at depths shallower than 40 m to

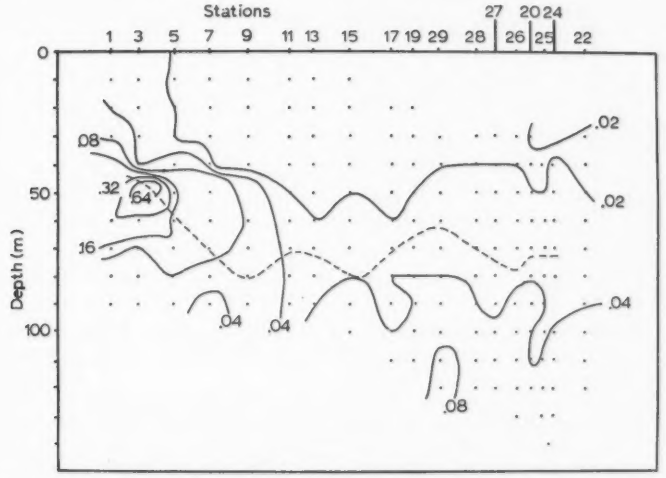

FIG. 2. Spatial distribution of chlorophyll *a*, μg/liter (*solid rule*) and the isolume of 1% of surface irradiance (*broken rule*).

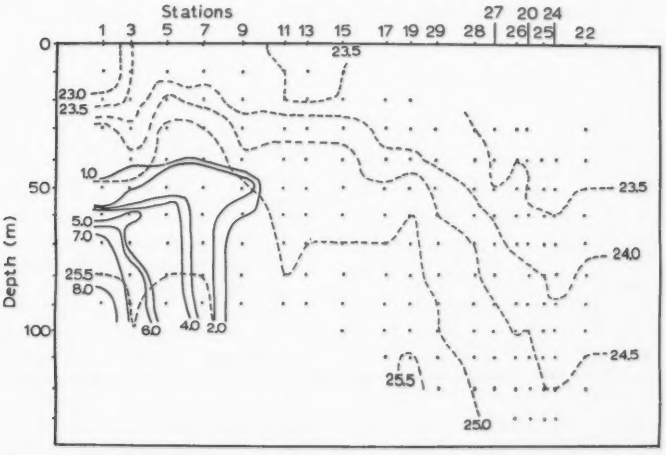

FIG. 3. Spatial distributions of NO_3-N + NO_2-N, μM (*solid rule*) and σ_t (*broken rule*).

6 μM at 75 m (Fig. 3). Seaward of station 7, chlorophyll *a* concentrations increased with depth over the depth interval sampled.

A maximum concentration of phytoplankton carbon occurred at station 4 extending in decreasing concentrations from 10 to 50 m (Fig. 4). At this station, phytoplankton carbon was dominated by the diatoms, *Asterionella japonica*, *Rhizosolenia* spp., and *Hemiaulus* sp. Concentrations of phytoplankton carbon decreased seaward of station 4 and they decreased with increasing depth. At these offshore stations, phytoplankton carbon was do-

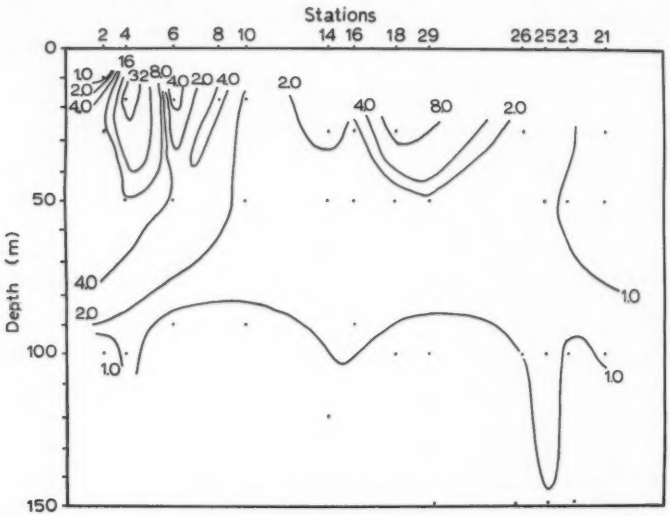

FIG. 4. Spatial distribution of phytoplankton carbon, μg/liter.

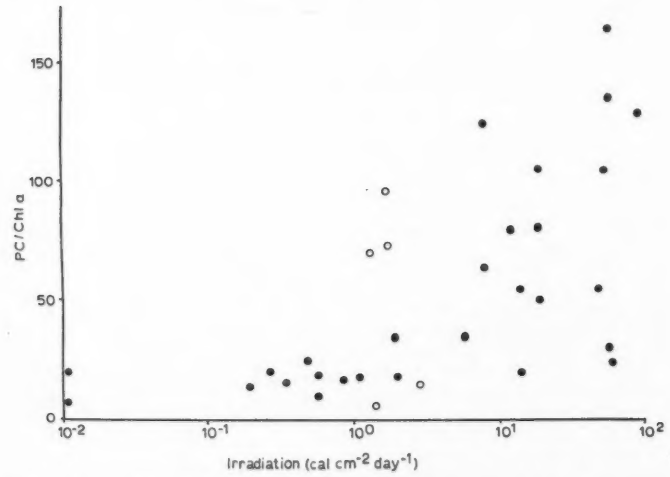

FIG. 5. Relationship between ratios of phytoplankton carbon to chlorophyll *a* and daily irradiations from CNAV *Endeavour* (●) and RV *T. G. Thompson* (○) stations.

TABLE 1. Compensation irradiations (CI),[a] carbon content (PC), and taxonomic composition of phytoplankton at five stations.

Station	Depth (*m*)	PC (*μg/liter*)	Taxonomic composition (% *PC*)	CI (*cal cm^{-2} day^{-1}*)
022	65	73	Diatoms — 70 Dinoflagellates — 26	0.5±0.7
025	38	6	μ-flagellates — 47 Dinoflagellates — 36 Diatoms — 17	0.5±0.5
028	60	10	Dinoflagellates — 83 μ-flagellates — 11	<1.2
036	30	167	Diatoms — 72 Dinoflagellates — 26	0.7±0.1
041	30	66	Diatoms — 76 Dinoflagellates — 18	0.5±0.5

[a]See text for estimation.

minated by small (5–15 μm in longest dimension) dinoflagellates and by small (2–10 μm in longest dimension) flagellated cells.

Estimates of ratios of phytoplankton carbon to chlorophyll *a* were made to provide information about errors incurred in determining phytoplankton carbon (Hobson et al. 1973). Ratios were determined for depths and stations where concentrations of phytoplankton carbon were estimated by dividing carbon concentrations by estimates of chlorophyll *a* based on isopleths of chlorophyll *a* (Fig. 3) because sampling positions for both measurements did not coincide. The variability and the magnitude of these ratios decreased as daily irradiations to which cells were exposed decreased (Fig. 5). Above a daily irradiation of 5 cal cm^{-2} ratios varied between 22 and 165 ($\bar{x}$ = 82). Three values varying between 270 and 456 and occurring at 15 m, stations 6 and 8, and at 25 m, station 18 were omitted. These ratios indicated that phytoplankton biomasses probably were overestimated at these depths and stations. Below a daily irradiation of 2 cal cm^{-2}, ratios varied between 8 and 35 ($\bar{x}$ = 19). However, the latter variation was too small when data from the *T. G. Thompson* stations were considered. One sample from each of 5 stations was taken from depths close to 1% light depths, and the phytoplankton carbon from three of these samples was dominated by diatoms (72–76%). Ratios of phytoplankton carbon to chlorophyll *a* varied between 70 and 98 ($\bar{x}$ = 81) in these three samples (Fig. 5).

Compensation irradiations were determined at each of the 5 aforementioned *T. G. Thompson* stations. Samples contained phytoplankton carbon varying between 66 and 167 μg/liter where crops were dominated by diatoms, and phytoplankton carbon varying between 6 and 10 μg/liter where crops were dominated by flagellated cells and dinoflagellates (Table 1). Principal diatom genera were *Chaetoceros* spp., *Rhizosolenia* spp., *Tropidonesis antarctica*, and *Melosira* spp. Also, unidentified pennate and centric diatoms represented a consider-

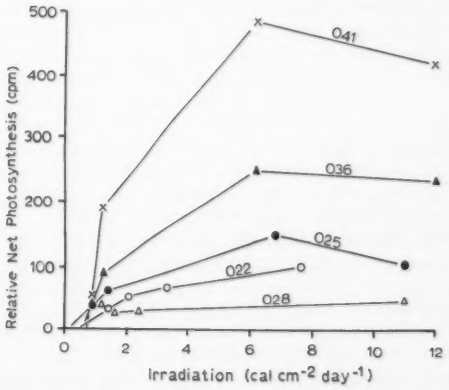

FIG. 6. Responses of net photosynthesis to daily irradiation by subsurface phytoplankton from five RV *T.G. Thompson* stations.

able amount of carbon. Compensation irradiations were estimated by extrapolating lines connecting the measured rates of net photosynthesis at the two lowest irradiations to which cells were exposed to net photosynthetic rates of zero (Fig. 6). Variability of compensation irradiations was estimated graphically by variability among replicate measurements of net photosynthesis made at the two lowest irradiations to which cells were exposed. Results (Table 1) indicated that compensation irradiations of phytoplankton varied between 0.5 and 0.7 cal cm^{-2} day^{-1}, but variability was large, giving estimates of compensation irradiations varying between 0 and 1.2 cal cm^{-2} day^{-1}. No separation of data could be made by taxonomic criteria. Assuming that a value of 0.7 cal cm^{-2} day^{-1} is a reasonable compensation irradiation, and assuming that this value can be extrapolated through time, then compensation depths for the Hawaii cruise increased from 50 m at station 1 to 90 m at station 7. Seaward of station 7, compensation depths varied between 90 and 105 m.

Discussion

The spatial distributions of chlorophyll *a* and phytoplankton carbon observed during this study indicate that chlorophyll *a* concentrations of cells increased relative to cell carbon as depth increased. Presumably this effect of depth on cell composition was due to decreasing daily irradiations to which cells were exposed as depth increased. This increase in chlorophyll *a* concentration of cells could be a physiological adaptation by cells to low daily irradiations to which they were exposed. This conclusion is supported by many observations of an inverse relationship between pigment concentrations of cells normalized on cell numbers and illuminance (Allen 1968; Brown and Richardson 1968; Paasche 1967; Sheridan 1972; Steemann Nielsen et al. 1962). No studies known to the authors have been carried out to determine whether or not this inverse relationship occurs when pigment concentrations are normalized on cell carbon. Ratios of phytoplankton carbon to chlorophyll *a* estimated by us decreased with decreasing daily irradiations to which cells were exposed, although variability among ratios was sometimes large. Most of the ratios were similar to those reported by others (Eppley 1968; Hobson et al 1973; Strickland 1965). The foregoing observations and others (Cahet et al. 1972; Hobson and Lorenzen 1972; Kiefer et al. 1972; Taniguchi and Kawamura 1972) indicate that subsurface chlorophyll *a* maxima can be due to either increases in cellular concentrations of chlorophyll *a* or to subsurface accumulations of cells, or to both.

The minimum daily irradiance that must be exceeded to allow positive net photosynthesis was estimated to be between 0.5 and 0.7 cal cm^{-2} for phytoplankton populations dominated by either diatoms or by μ-flagellates and dinoflagellates. The technique employed by us does not give precise measurements of compensation irradiations. However, we know of no other method that will provide more precise measurements than we obtained. Our values are similar to those determined by Anderson (1969) and by Thomas (1966) for *Chaetoceros* sp., and are lower than those estimated by Jenkin (1937) for *Coscinodiscus excentricus* and by Thomas (1966) for *Gymnodinium* spp. The former values suggest that compensation depths were between 10–20 m lower than depths at which 1% of the surface irradiation occurred in summer of 1971 in the northeast Pacific Ocean.

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NOTES

Three-Dimensional Computer Graphics in Fisheries Science[1]

DANIEL J. BROWN AND LOH LEE LOW

Center for Quantitative Science in Fisheries, Forestry, and Wildlife
University of Washington, Seattle, Wash. 98195, USA

BROWN, D. J., AND L. L. LOW. 1974. Three-dimensional computer graphics in fisheries science. J. Fish. Res. Board Can. 31: 1927–1935.

These illustrations of three-dimensional computer-generated graphics depict the emergence of definable areas of fisheries science where computer-drawn images yield a visual feeling for the behavior of multidimensional variables. The images are perspective and orthographic projections of a three-dimensional array of connected points which are used to present the results of computer analysis, thus reducing voluminous amounts of complex data into more easily understood visual images. The examples collected here transform information from computer models into conceptual form for humans.

BROWN, D. J., AND L. L. LOW. 1974. Three-dimensional computer graphics in fisheries science. J. Fish. Res. Board Can. 31: 1927–1935.

Ces graphiques à trois dimensions tracés par ordinateur signalent l'émergence de champs définissables, au domaine des sciences des pêches, dans lesquels des images produites par un ordinateur évoquent une sensation visuelle du comportement de variables à plusieurs dimensions. Les images sont des projections orthographiques et en perspective d'un assemblage à trois dimensions de points reliés entre eux, et qui sont utilisées pour présenter les résultats de l'analyse par ordinateur; quantités de données complexes sont ainsi réduites à des images visuelles plus facilement comprises. Les exemples réunis ici montrent comment les renseignements fournis par des modeles d'ordinateur sont transformés en une forme conceptuelle pour les humains.

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THE following collection of illustrations introduces to fisheries science a three-dimensional computer technique to visually simulate biological processes and visually analyze statistical fisheries data. The images assist the data analyst in conceptualizing thoughts, formulating ideas, and conveying multidimensional information for presentation to other people. They allow man to interact with the computer in a language that is powerful and appealing.

The most basic data and models of biological systems require an understanding of the behavior of variables in three-dimensional space and time. This applies to: fish behavior, survey biomass calculations, and catch–effort data analysis. The Japanese Bering Sea data and the International Pacific Halibut Commission survey of the southeastern flats of the Bering Sea are examples of spatial and temporally distributed fisheries data. Other more mathematically abstract applications which yield a feeling for the sensitivity of several variables are presented as well as graphical depiction of the equilibrium yield per recruit principle and some age–length frequency data.

Spatial, temporally distributed statistics — A very basic type of fishery statistics collected by commercial fisheries or exploratory vessels is the catch–effort statistics designated by gear type, vessel type, species, and location. The display of catch or effort levels by geographical locations presents a clear picture of catch or effort levels. An example of catch levels by location (Fig. 1) shows the predominant areas where walleye pollock (*Theragra chalcogramma*) was caught by the Japanese mothership fishery in the Bering Sea for the combined years of 1963 through 1971.

A series of geographical catch graphs can reveal the dynamic nature of a fishery (Fig. 2). The series

[1]Contribution No. 406, College of Fisheries, University of Washington, Seattle, Wash. 98195, USA.

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shows relative amounts of total groundfish taken by the Japanese mothership fishery in the Bering Sea from 1964 through 1971.

Figure 3 depicts the bathymetry of the Bering Sea floor for the same geographical area as Fig. 1. Figure 3a indicates the 500-m depth contours and Fig. 3b the three-dimensional perspective projection of the same area. Note that the catch of walleye pollock (Fig. 1 and 2) follows the edge of the continental shelf.

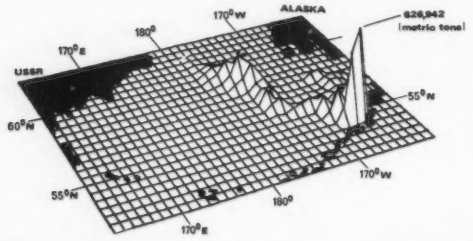

FIG. 1. Geographical distribution of total walleye pollock (*Theragra chalcogramma*) catch by the Japanese mothership and independent trawl fisheries in the Bering Sea for 1963 through 1971 combined. (Data Source: National Marine Fisheries Service)

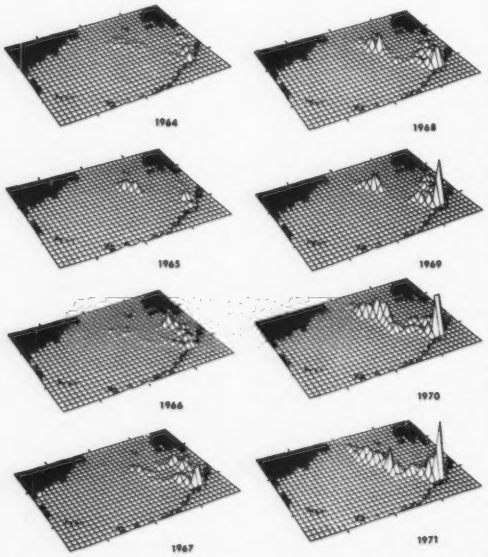

FIG. 2. Time-series graphs showing geographical distribution of annual total groundfish catch by the Japanese mothership, longline–gillnet and independent trawl fisheries in the Bering Sea, 1964 through 1971. (Data Source: National Marine Fisheries Service)

An impressive feature of this method is the ability to summarize 12,000 records (80 BCD characters per record) in the data base speedily and accurately for a quick visual interpretation on the dynamic nature of the fishery. At the same time, figures for catch and effort can also be printed out in arrays to complement graphic presentations.

With proper modifications in the program used to compute desired statistics, any combinations of three-dimensional spatial–temporal graphs by gear types or species can be plotted.

Figure 4 shows the results of three surveys (Best 1970) conducted by the International Pacific Halibut Commission displayed as a computer mosaic. Catch in number of fish is projected for 1965, 1967, and 1972. Figure 5 depicts various mosaics constructed for the 1965 survey.

Age and length frequency statistics — A very common presentation of age and length frequency statistics is to plot frequency (or percent frequency) of a species of fish on the Y axis against age or length of the fish on the X axis (Fig. 3 of Westrheim 1973). An alternative method is to combine the two graphs into one three-dimensional image.

Figure 6 shows an image constructed from samples of male and female Pacific hake (*Merluccius gayi*) taken off the coast of Chile and analyzed by Ehrhardt (1973a). Several features of interest can be easily extracted. The plots show that female hake live longer than the males which do not show up at age 6. Females are also longer in length than the males for the same age-class. The solid lines running through the length peaks for each age-class grow closer together as the fish grow older, reflecting decreasing growth rates with age as will be predicted by the von Bertalanfy equation (Beverton and Holt 1957).

Yield per recruit surface — The equilibrium yield per recruit principle is an extremely valuable concept that makes use of parameters of growth, natural mortality (M), fishing mortality (F), and age of recruitment of an exploited fish stock to predict effects that changes in fishing strategy may have upon biomass yields from a given cohort.

Yield surfaces (Fig. 7, 8, and 9) can be constructed from numerical solutions of yield models as were done by Ehrhardt (1973b) using the Beverton and Holt (1957) yield model programmed by Paulik and Gales (1964). Figure 7 demonstrates the ability to select viewing position by showing four views of the yield surface for male hake off Chile at $M=0.40$ and variable rates of F and age of entry. The views represent the surface visualized from four different points in space, as referenced from

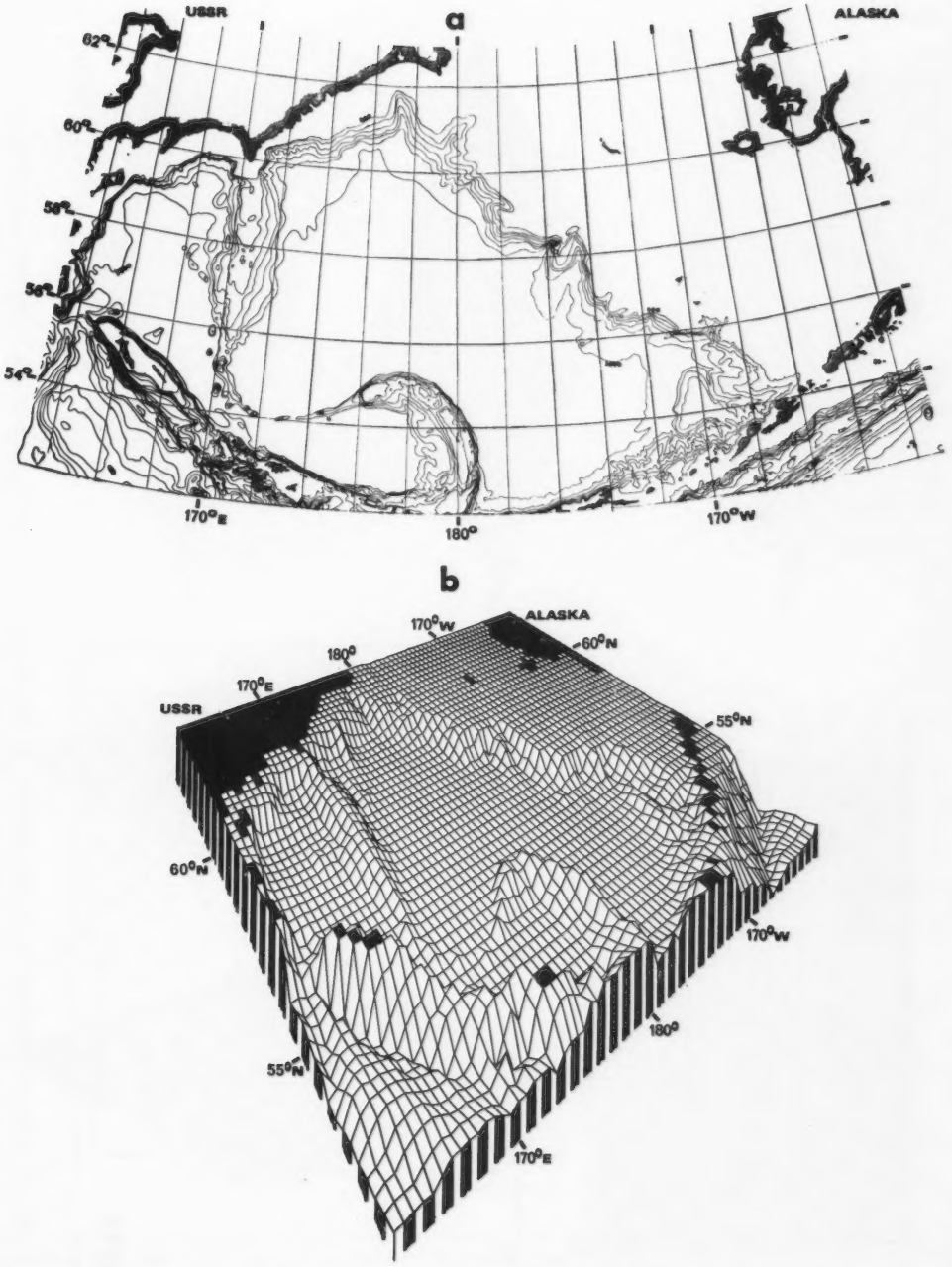

FIG. 3. Bathymetry maps of the Bering Sea. a, Contour map with 500-m contour intervals and b, three-dimensional perspective projection.

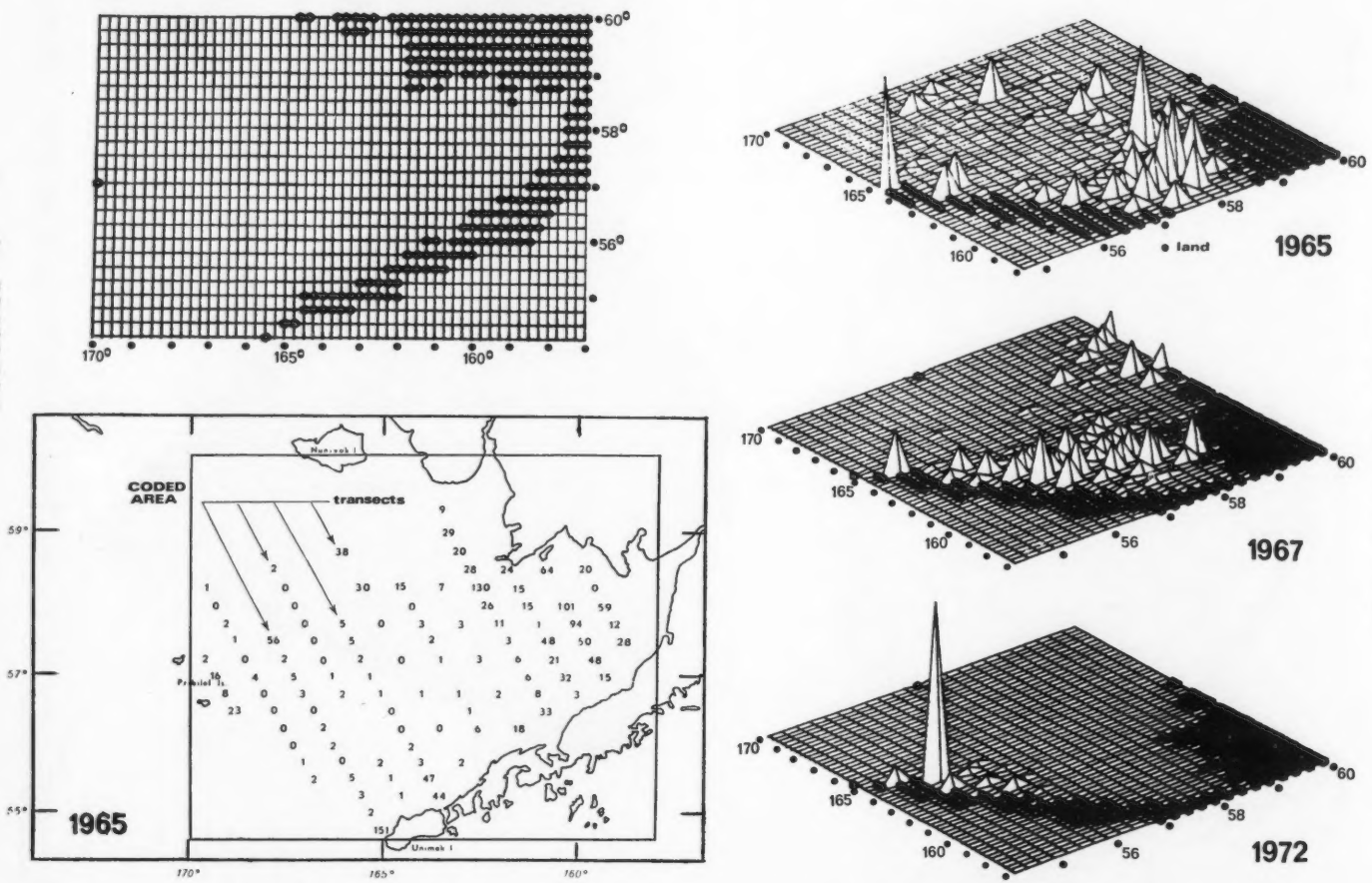

FIG. 4. Catch per 60-min haul by the M/V *Tordenskjold*, June–August 1965, 1967, 1972. (International Pacific Halibut Commission)

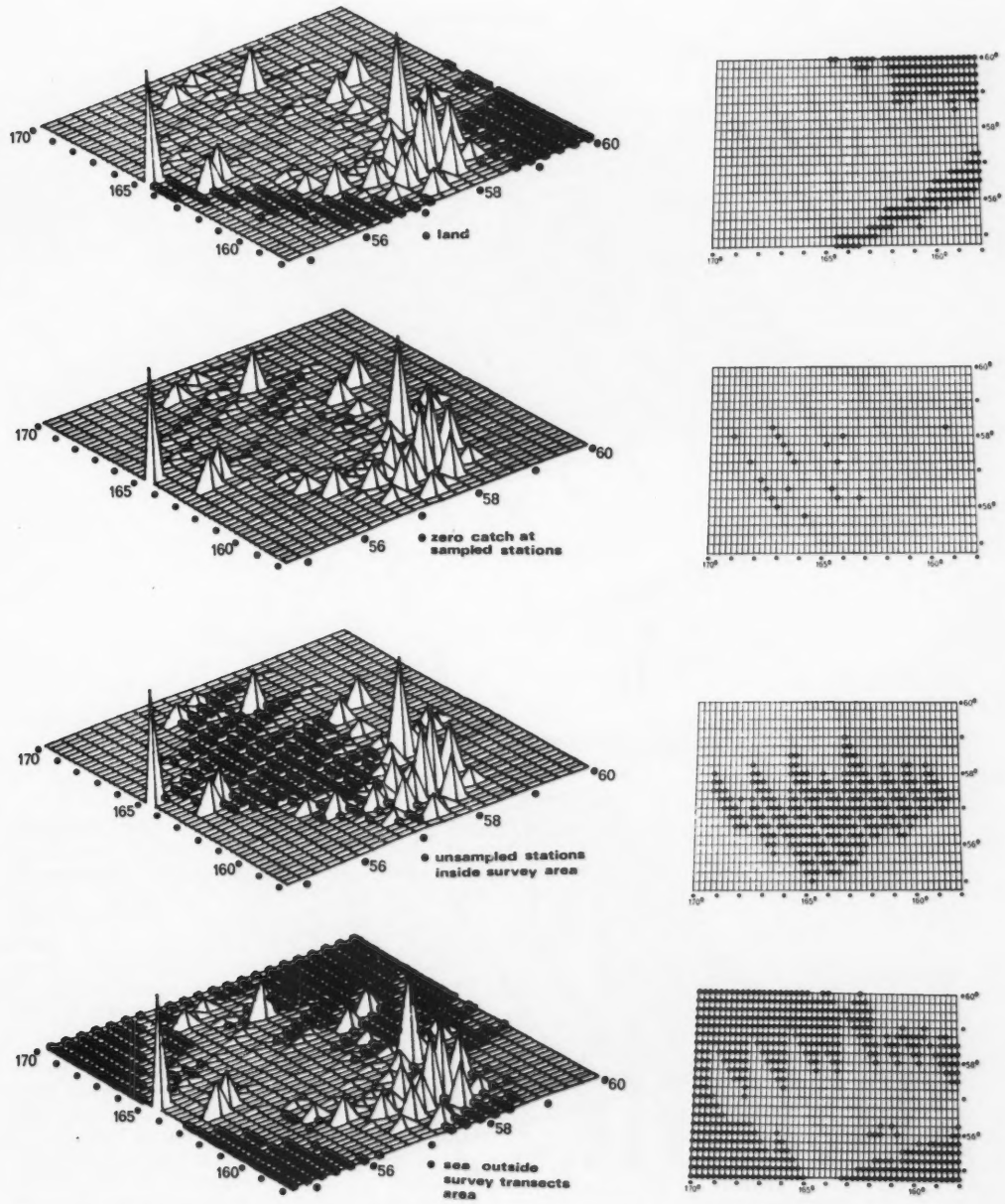

FIG. 5. Depiction of halibut catch from 45° sampling transects in the southeastern flats of the Bering Sea. Shaded onto the grid are coded points for various characteristics of the mosaic. Peaks represent catch per 60-min haul by the M/V *Tordenskjold*, June–August 1965.

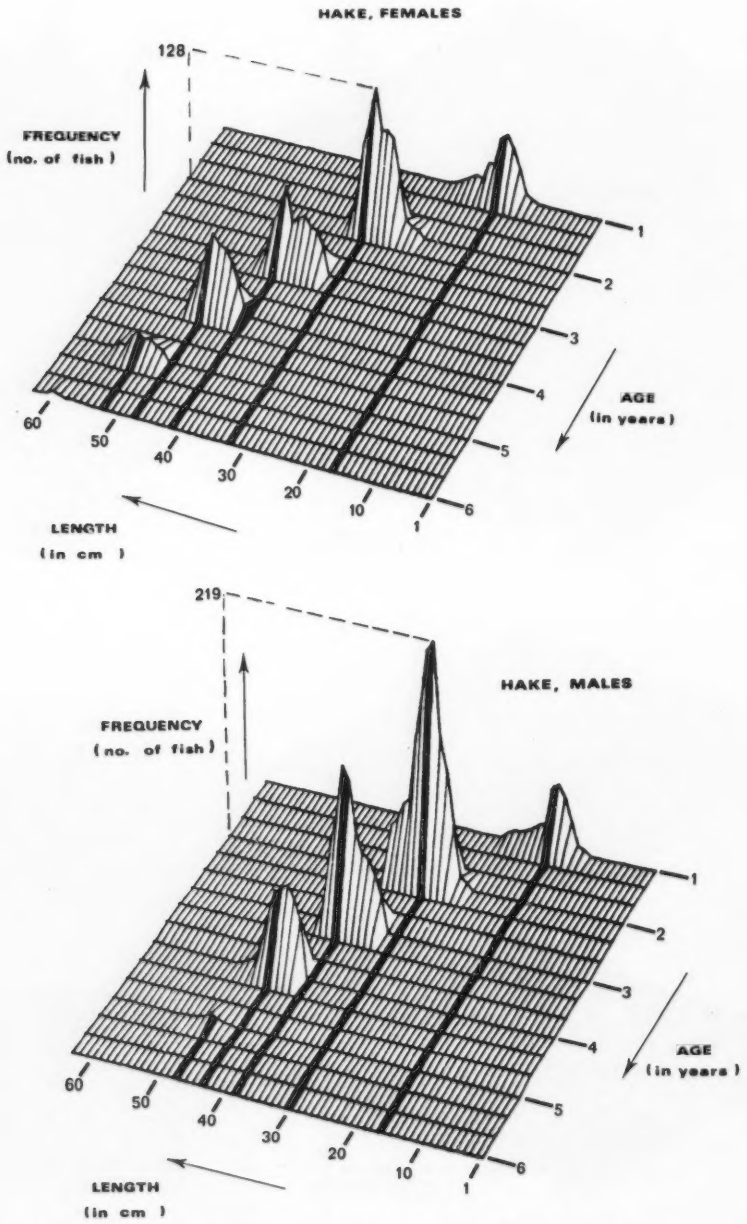

FIG. 6. Age–length frequency graphs of male and female Pacific hake (*Merluccius gayi*).

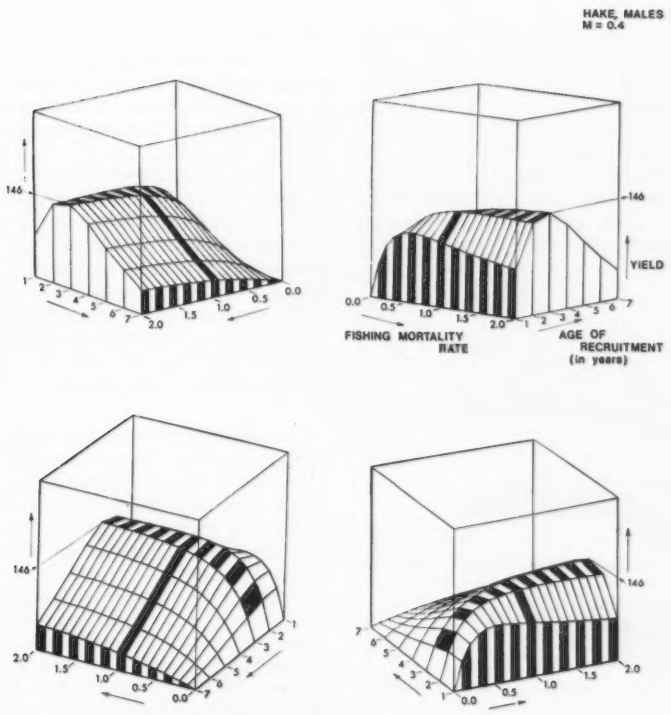

FIG. 7. Four views of a surface showing equilibrium yield for male hake at an instantaneous natural mortality rate of 0.4.

the point of origin of the X, Y, and Z axes. Suggested age of entry for maximum yield is at age 3 with an instantaneous fishing mortality of 0.9. Figure 8 is an orthogonal view of the female hake equilibrium yield surface. It is a view down the age of recruitment axis to eliminate its dimension. The figure gives a better idea of maximum equilibrium yields at an instantaneous mortality rate of 0.4, varying rates of fishing intensities, and ages of recruitment.

Once a good three-dimensional view is established, natural mortality rates or other parameters in the yield model can be changed to study equilibrium yield responses with each change. Figure 9 shows hypothetical response surfaces for the English sole (*Parophrys vetulus*) off the coast of Washington with varying natural mortalities between 0.1 and 0.35. The peak of the surfaces (darkened area) are of interest for management decisions. With increasing natural mortalities, equilibrium yields are shown to drop as expected and the age of entry should also be lowered.

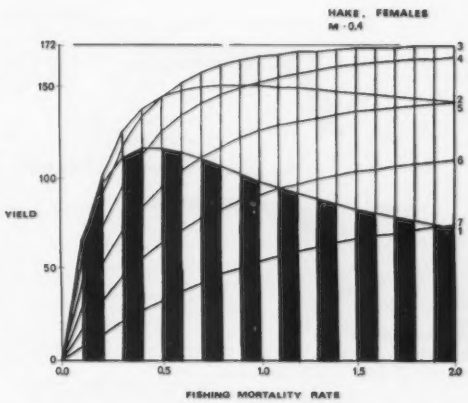

FIG. 8. A two-dimensional view of equilibrium yield per recruit levels for ages 1–7 female hake at variable instantaneous fishing mortality rates and a constant instantaneous mortality rate of 0.4.

Theoretical abstractions and models — Three-dimensional computer graphics can be invaluable for research on theoretical models where quick interpretation of large volumes of output is necessary. The technique was used in a water temperature model by Fowler (1973). Temperatures predicted by the model are graphed (Fig. 10) for an average year using weekly means of air temperature and total solar insolation. Two views of the results are shown where the highest temperature (22.4 C) occurs during the summer weeks at the surface while the highest bottom temperature (15.3 C) lags behind a few weeks.

The front view of the graph not only shows the gradual warming trend of the lake as a whole, but also the gradual thermal stratification of the lighter warmer waters of the upper layers from the denser, cooler, and deeper waters. This phenomenon is illustrated by the curved lines showing temperature at depth for any given time. The rear-view graph depicting the cooling trend of the lake shows that thermal stratification changes to an even water temperature distribution as shown by the straight lines parallel to the depth axis.

The model-predicted temperature graphs are very similar to the graphs plotted from real data and quick comparisons will give the modeler an idea of the validity of the model.

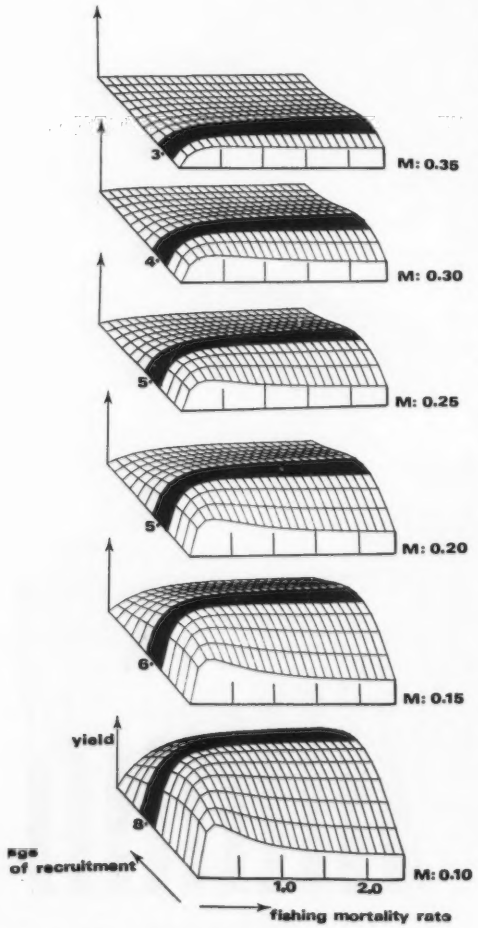

FIG. 9. Equilibrium yield per recruit surfaces for English sole (*Parophrys vetulus*) at instantaneous natural mortality rates ranging from 0.1 to 0.35 and instantaneous fishing mortality rates from 0 to 2.5.

Computer hardware and software — The PICTURE program modified from an original program from Los Alamos Scientific Laboratory, New Mexico which produced the images presented herein is available upon request. It is written in FORTRAN IV for a CDC6400 computer. For several reasons which will not be dealt with here, the program is not readily transferable to other machines. There are, however, other programs which project similar images that are locally available at computer centers.

The NORFISH staff and students make extensive use of a Tektronix remote display terminal model T4010 and the CDC6400 remote terminal system INTERCOM[2] which generated these images.

Acknowledgments — We are indebted to Dr M. L. Prueitt of the Los Alamos Scientific Laboratory for the original PICTURE program and Drs L. J. Bledsoe and G. S. Morishima who reviewed the manuscript and offered many helpful suggestions throughout this work. We also wish to thank Dr D. L. Alverson and Mr A. T. Pruter for providing Japanese fishery catch statistics collected by the Fishery Agency of Japan. Research was supported under the NORFISH project of the University of Washington Sea Grant Program and the National Marine Fisheries Service, National Oceanic and Atmospheric Administration, U.S. Department of Commerce.

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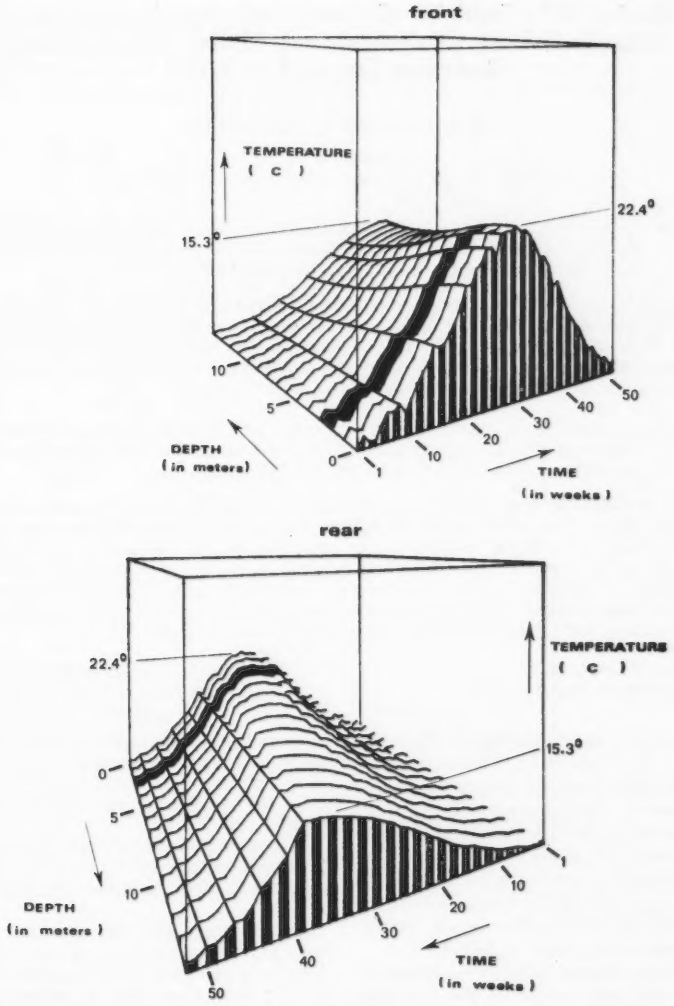

FIG. 10. Two views of Fern Lake temperature profile predicted from a theoretical model.

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Retarded Behavioral Development and Impaired Balance in Atlantic Salmon (*Salmo salar*) Alevins Hatched from Gastrulae Exposed to DDT

P. A. DILL AND R. C. SAUNDERS

Department of Biology
St. Francis Xavier University, Antigonish, N.S. B0H 1C0

DILL, P. A., AND R. C. SAUNDERS. 1974. Retarded behavioral development and impaired balance in Atlantic salmon (*Salmo salar*) alevins hatched from gastrulae exposed to DDT. J. Fish. Res. Board Can. 31: 1936–1938.

Eggs of Atlantic salmon (*Salmo salar*) were subjected to 0.1, 0.05, 0.01, or 0.005 ppm DDT at gastrulation. Behavior of hatched fish was observed in DDT-free aquaria for about 30 days, and compared with that of controls. Exposure to the two higher concentrations of DDT retarded behavioral development and impaired balance. The consequences of such behavior changes to wild fish are discussed.

DILL, P. A., AND R. C. SAUNDERS. 1974. Retarded behavioral development and impaired balance in Atlantic salmon (*Salmo salar*) alevins hatched from gastrulae exposed to DDT. J. Fish. Res. Board Can. 31: 1936–1938.

Nous avons exposé des oeufs de saumon atlantique (*Salmo salar*) à des concentrations de DDT de 0.1, 0.05, 0.01 ou 0.005 ppm au moment de la gastrulation. Nous avons ensuite observé le comportement des poissons après éclosion, dans des aquariums dépourvus de DDT, pendant environ 30 jours. Nous avons comparé leur comportement avec celui de témoins. L'exposition aux deux plus fortes concentrations de DDT retarde le développement du comportement et affaiblit le sens de l'équilibre. Nous analysons les conséquences de tels changements de comportement pour des poissons sauvages.

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THE effect of DDT on fish populations in rivers was initially thought of primarily in a lethal context. It is now known that sublethal concentrations of DDT cause sensory and/or motor impairment (Ogilvie and Anderson 1965; Anderson and Peterson 1969; Anderson and Prins 1970; Davy et al. 1972) which could lead to a gradual and less dramatic reduction in fish populations. In future, behavioral indicators of environmental toxins should supplement or replace estimators such as LD50.

In Atlantic salmon (*Salmo salar*), impairment of behavior during the early stages in the life cycle would be useful as an indicator because the younger the fish, apparently, the more serious the effect of DDT (Elson 1967). However, our knowledge of behavior in very young salmon (alevins) is limited (White 1915; Dill 1970) and the effects of sublethal environmental pollutants on their behavior has not been investigated. In this study, Atlantic salmon eggs were exposed to sublethal concentrations of DDT and the subsequent development of alevin behavior was observed and compared with that of controls.

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Materials and methods — Atlantic salmon eggs were obtained from late run migrants of the Philip River, N.S. They were incubated in groups of 150 in glass trays (30 × 20 × 5 cm) containing aerated spring water at 10 ± 3 C.

At gastrulation (determined by the authors to be 11 days after fertilization) each tray was drained and refilled with 1000 ml of the following solution:

Tray 1: spring water only (control), pH 5.7
Tray 2: 100 ppm acetone in spring water
Tray 3: 0.005 ppm DDT in acetone and spring water
Tray 4: 0.01 ppm DDT in acetone and spring water
Tray 5: 0.05 ppm DDT in acetone and spring water
Tray 6: 0.10 ppm DDT in acetone and spring water

DDT was extracted from 50% DDT wettable powder (Nichols Chemical Co.) by treatment with cyclohexane, filtration, and evaporation.

The eggs remained in these solutions until hatching (approximately 1 mo). Upon hatching, 10 fish from each tray were transferred to glass aquaria (35 × 23 × 18 cm high) containing continuously aerated spring water (15 cm deep) only. The fish were maintained at 16 ± 0.5 C, in a 12L:12D photoperiod.

Fish behavior was observed for 10 min during mid-photophase, every 3 or 4 days. At the outset of each 10-min observation period, the body position of each

alevin was classified as: on-side or upright. Upright position was further divided into: plough position — ventral surface of yolk sac in contact with substrate, anterior–posterior axis approximately 45° off horizontal with head down, or bottom-swim position — ventral surface of yolk sac in contact with substrate, anterior–posterior axis horizontal.

During the observation period, changes between on-side and upright position were recorded as right-fall behavior. Also, the locomotor behavior of alevins

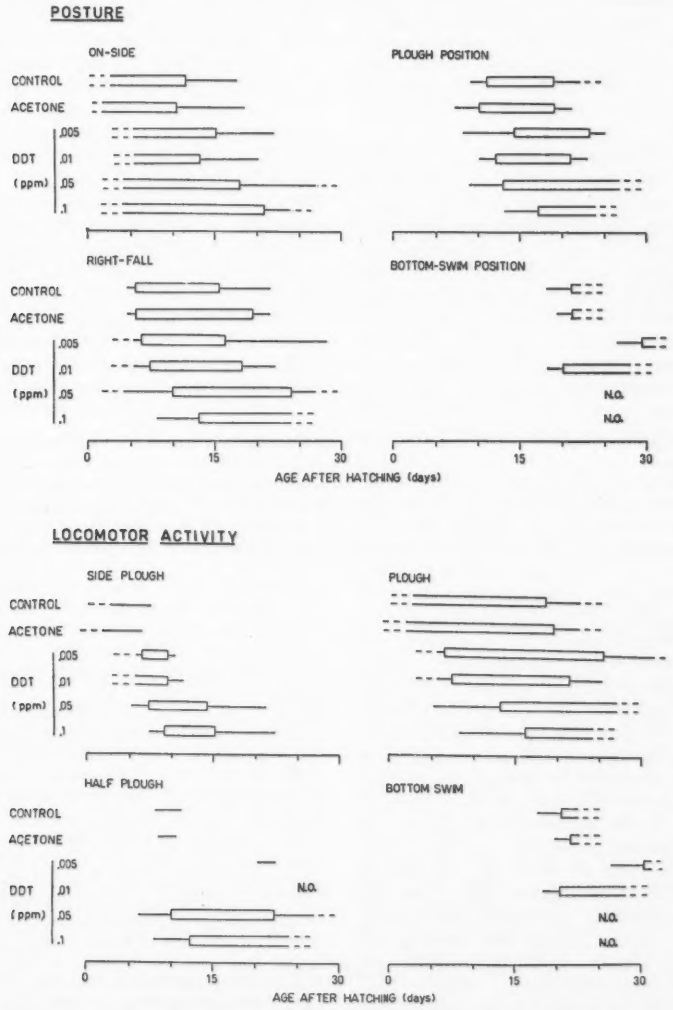

FIG. 1. Ontogeny of postures and locomotor activity for Atlantic salmon (*Salmo salar*) alevins subjected to varying concentrations of DDT in acetone. Horizontal lines express the range over which a particular act was performed. Open horizontal bars indicate the age when more than 4 of 10 fish performed an act. Dotted lines indicate when an act was observed at the beginning or end of the observation age. Therefore, the initiation or termination of that act was not known. Acts never observed are labelled N.O.

was observed and classified as either side-plough — vigorous body and tail movements resulting in circular locomotion of about 2-cm radius on the substrate in the on-side position, or half-plough — vigorous body and tail movements resulting in circular (3–8-cm radius) or forward locomotion on the substrate in a position midway between on-side and plough positions, or plough — vigorous body and tail movements resulting in forward locomotion on the substrate in the plough position, or bottom-swim — vigorous body and tail movements resulting in forward locomotion on the substrate in the bottom-swim position.

Results and discussion — Exposure of Atlantic salmon eggs to 100 ppm acetone at gastrulation did not appear to influence the normal ontogeny of alevin behavior. Newly hatched alevins treated with acetone and spring water or both were usually on their sides. Soon they began a righting reflex and when upright they locomoted in plough position. Just prior to leaving the substrate (in gravel-free environment) they adpoted an upright bottom-swim position and locomoted in a bottom-swim. In the natural environment they may leave the gravel at any time up until they absorb their yolk sac. For details on the ontogeny of alevin behavior see Dill (1970).

The general effect of DDT was to delay the appearance of behavior patterns (Fig. 1). This delay became more pronounced at the higher treatment levels, e.g. the onset of right-fall and plough behavior was delayed more as the concentration of DDT increased. In some cases, treated fish never displayed behavior exhibited by control fish (N.O. in Fig. 1). The transition from plough position to bottom-swim position and from plough to bottom-swim locomotion were perhaps reflections of the relation of yolk-sac size to degree of growth (Dill 1970). Retardation of both of these transitions suggests that DDT may reduce the rate of yolk-sac absorption by reducing activity. However, in treated fish, the late starting age of right-fall behavior, and the persistence of right-fall and on-side behavior, suggests that the DDT slowed down the growth rate of the alevins.

DDT may also have an effect on the balance mechanism of fish. DDT-treated fish, especially at higher concentrations, performed side-plough and half-plough behavior. These were seldom performed by control fish (Fig. 1). These two abnormal acts, side-plough and half-plough, plus a high frequency of right-fall behavior in treated fish suggests vestibular damage. Such an impairment was not mentioned by earlier workers studying sublethal effects of DDT on the nervous system (Anderson 1968; Aubin and Johansen 1969; Anderson and Peterson 1969; Anderson and Prins 1970; Davy et al. 1972). Sublethal behavior changes may be of use as indicators of environmental toxins. Abnormal behavior, such as side-plough and half-plough, would be more reliable as indicators of toxicity than would changes in the timing of ontogenetic events. In addition, the rather long time between DDT exposure and the observation of abnormal behavior emphasizes that even if immediate changes do not occur one should not rule out the possibility of latent changes.

Modifications in normal behavior would have their first effect on salmon populations during emergence from gravel. DDT-treated wild fish exhibiting behavior similar to that observed in this experiment would be incapable of "swim-up" behavior (Bams 1969; Dill 1970) and surface-oriented locomotion necessary to get out of the gravel (Dill 1970). Postponement of emergence, past its normal age, could result in alevin starvation because emergence typically occurs at the time when yolk sac reserves are almost exhausted (Dill 1970). If the alevins did emerge they would have considerable difficulty feeding and holding position in a favorable habitat, and would be extremely vulnerable to predation.

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Serological Diagnosis of Corynebacterial Kidney Disease of Salmonids

PETER K. CHEN

Department of Biology, Georgetown University, Washington, D.C. 20007, USA

G. L. BULLOCK, H. M. STUCKEY, AND A. C. BULLOCK

U.S. Fish and Wildlife Service, Eastern Fish Disease Laboratory, Kearneysville, W. Va. 25430, USA

CHEN, P. K., G. L. BULLOCK, H. M. STUCKEY, AND A. C. BULLOCK. 1974. Serological diagnosis of corynebacterial kidney disease of salmonids. J. Fish. Res. Board Can. 31: 1939–1940.

An immunodiffusion test is described which provides a rapid, sensitive, and definitive diagnosis of corynebacterial kidney disease of salmonid fishes. Specific precipitin lines are formed within 24 h when rabbit antiserum is reacted with kidney disease cell suspensions or internal tissue homogenates containing the specific bacterium. No precipitin lines are formed with tissue homogenates from healthy fish or from those injected with other fish pathogenic bacteria.

CHEN, P. K., G. L. BULLOCK, H. M. STUCKEY, AND A. C. BULLOCK. 1974. Serological diagnosis of corynebacterial kidney disease of salmonids. J. Fish. Res. Board Can. 31: 1939–1940.

Les auteurs décrivent une épreuve d'immunodiffusion qui fournit un diagnostic rapide, sensible et final de la maladie corynébactérienne des salmonidés. Il se forme des lignes de précipitine spécifiques en dedans de 24 h, quand on fait réagir l'antisérum de lapin avec des suspensions de cellules de rein malade ou avec des homogénats de tissu interne contenant la bactérie spécifique. Il ne se forme pas de lignes de précipitine avec des homogénats de tissu de poissons sains ou inoculés avec d'autres bactéries pathogènes de poisson.

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THIS report describes an immunodiffusion technique for diagnosing overt cases of corynebacterial kidney disease (KD) in salmonids. Kidney, liver, blood, or spleen from diseased fish is used, and the KD antigen can be detected in these tissues within 24 h. This procedure offers a more reliable diagnosis of KD than the one usually used, that of observing small gram-positive diplobacilli in infected tissues. Microscopic diagnosis of KD may be fallible since other gram-positive bacteria having the same morphology as the KD bacterium occur in fish tissue (Bullock et al. 1971; Bullock and Snieszko 1969; Collins 1970).

The basis of the immunodiffusion test is detection of soluble KD antigen in infected tissues. The following procedures were carried out to determine the reliability of this test.

Trout used as sources of tissues for assay were either naturally or experimentally infected with KD. Naturally infected trout were obtained from hatchery epizootics of KD in Michigan and Pennsylvania. For experimental infections, brook trout (*Salvelinus fontinalis*) 15–20 cm in total length were given intraperitoneal injections of 4, 8, 12, or 20 $\times$ 10^9 viable KD cells suspended in saline. Additional trout were injected with: 20 $\times$ 10^9 killed KD cells, saline alone, viable *Aeromonas salmonicida* cells, or viable cells from a gram-positive bacterium isolated from fish, which unlike the KD bacterium grew rapidly on media without added cysteine. All injected trout were held in 12.5 C spring water, the chemistry of which has been reported (Warren 1963). Heart, liver, spleen, and kidney tissues were assayed from experimentally infected trout, while kidney, liver, and blood (collected by the method described by Bullock and Snieszko 1969) were assayed from the naturally infected trout.

Immunodiffusion tests were carried out on tissues from 70 trout in 60-mm culture dishes each containing 6 ml of the following medium: 1% Noble agar, saline, and 0.01% Merthiolate(R). All wells

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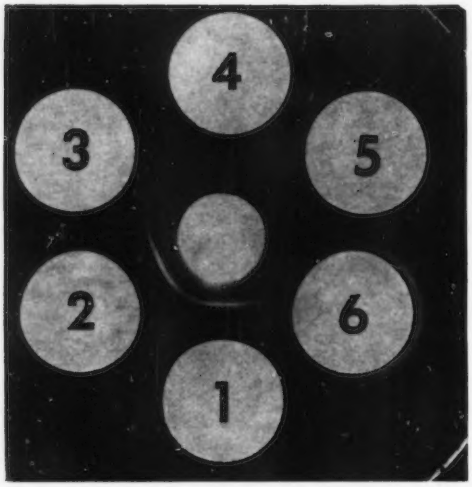

FIG. 1. Precipitin band(s) formed between rabbit anti-kidney disease serum (central well), kidney disease cell suspension (well 1), and renal homogenates containing the kidney disease bacterium (wells 2, 3). Normal renal tissue homogenate (well 4) and those infected with an unidentified (nonkidney disease) gram-positive rod (well 5) or *Aeromonas salmonicida* (well 6) failed to react.

were 6 mm in diameter and margins were 6 mm apart. The center well was charged with 0.1 ml rabbit anti-KD serum (prepared by Microbiological Associates, Inc., Rockville, Md.) while peripheral wells contained the same amount of blood, 50% homogenates of liver, spleen, or kidney in Hanks' balanced salt solution, or KD cell suspension. Test dishes were kept moist, at 22±4 C, and examined daily for 4 days.

Organ homogenates from both experimentally and naturally infected trout and blood from the latter produced strong precipitin bands with rabbit anti-serum within 24 h. Based on the number of reactors and the speed of reaction, kidneys and liver were the preferred organs from both experimentally and naturally infected trout. The specificity of the reactions was shown by formation of a band (or bands) of identity between the specific antiserum, corynebacterial culture, and tissue homogenates from KD infected trout (Fig. 1). No bands were formed with tissues from healthy trout or from trout infected with the unidentified gram-positive bacterium or with *A. salmonicida*. Furthermore, no precipitin bands formed with tissue homogenates from trout injected with saline or heat-killed kidney disease bacterium.

Our studies indicate that by using the described Ouchterlony immunodiffusion test it is possible to detect and to identify antigens of the KD bacterium in internal tissues of salmonids with overt KD. Consequently, the protracted cultural technique previously needed to confirm a diagnosis of KD can now be avoided. This test is both rapid (results are obtained in 24 h) and definitive.

Since previous studies (Bullock et al. 1974) indicated that strains of the KD bacterium from different geographic locations of North America are serologically homogeneous, antiserum against any authentic KD culture may be used in the immunodiffusion test. The test works well using tissues from fish with over TKD — however, it remains to be determined whether the procedure is sensitive enough to detect fish covertly infected with the KD bacterium.

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Social Conditions Increase Intermolt Period in Juvenile Lobsters *Homarus americanus*

J. STANLEY COBB AND GEORGE R. TAMM

Department of Zoology
University of Rhode Island, Kingston, R.I. 02881, USA

COBB, J. S., AND G. R. TAMM. 1974. Social conditions increase intermolt period in juvenile lobsters. J. Fish. Res. Board Can. 32: 1941–1943.

Fifth and eleventh stage lobsters held in pairs showed significantly longer time between molts than did controls reared individually. In pairs the first animal to molt did not delay ecdysis in the fifth stage, but delayed up to 32% of the control molt period in the eleventh stage. In the fifth stage the second lobster to molt delayed up to 32% and in the eleventh stage up to 71%. Similar differences in time between molts were seen in both an open and a recirculating seawater system.

COBB, J. S., AND G. R. TAMM. 1974. Social conditions increase intermolt period in juvenile lobsters. J. Fish. Res. Board Can. 32: 1941–1943.

Des jeunes homards aux stades cinq et onze du cycle d'intermue, élevés en paires, ont montré un accroissement significatif de la période d'intermue par rapport à des animaux témoins élevés seuls. Dans une paire, le homard qui mue le premier ne retarde pas son ecdysie au stade cinq, mais au stade onze, il la retarde d'une durée atteignant 32% de la période d'intermue des témoins. Au stade cinq, le retard de l'ecdysie chez le second homard à muer atteint 32%, et 71% au stade onze. Des résultats semblables ont été obtenus aussi bien en eau de mer courante qu'en circuit fermé.

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WHEN held communally lobsters' aggressive behavior leads to mortality and physical damage. Present aquaculture methods involve physical isolation to circumvent this problem, but this is costly and space demanding. Little research has been done on the social aspect of communal rearing. Social conditions (Bliss and Boyer 1965; Aiken 1969; Cobb 1970), as well as hormonal conditions (Passano 1960) have been shown to affect the molting of decapod crustaceans. Previous work has shown that one of a pair of larval lobsters will delay its molt as compared to the first lobster to molt, or a control (Cobb 1970). Stewart and Squires (1968) showed that adult male lobsters held in crowded conditions showed a significant molt delay as compared to free living males. The purpose of the experiments reported here was to determine whether or not the delay seen in larval lobsters is also found in juveniles.

Materials and methods — Fourth stage lobster larvae obtained from the Massachusetts Lobster Hatchery at Oak Bluffs were held individually until they molted into the fifth stage, when they were either isolated (control) or paired (experimental) in either an open or a closed seawater system. Observations were made twice daily on molt condition, physical condition, and general behavior. The number of days between molts was counted for each animal. Treatments were arranged in a randomized block design, to examine the possible effects of extraneous environmental factors such as water flow and light intensity. Ninety-six pairs and 92 controls were started in the closed system, and in the open system 102 pairs and 94 controls were started. Likewise, after molting into the eleventh stage, animals were either paired or held individually in a completely randomized design. In this experiment there were 10 controls and 10 pairs. Data from fifth stage lobsters were analyzed by analysis of variance for unbalanced data. Data for eleventh stage lobsters were analyzed by a Mann–Whitney U test because of the small sample size and missing values. Analyses were performed at a rejection level of $\alpha = .05$.

In the fifth stage experiments, lobsters were reared in opaque plastic freezer cups of two sizes. Animals held individually were in square containers 8.4 cm on a side and 6.3 cm deep (70 cm² bottom area). Pairs of animals were held in containers 8.4 × 19.0 cm × 6.3 cm deep (160 cm² bottom area). Eleventh stage lobsters were reared in white acrylic partitioned trays. The dimensions of each compartment were 12.7 × 25.4 cm (322 cm² bottom area) for individuals and 25.4 × 25.4 cm (645 cm² bottom area) for pairs.

The open seawater system drew water directly from Narragansett Bay. Water was filtered through a rapid sand filter, a biological filter bed of gravel and dolomite

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chips, and through a fiber cartridge filter. Water was delivered to each container individually. Paired animals received twice as much water as the control. Water pressure was maintained by a standpipe so a uniform amount of water was delivered to each animal regardless of position within the water table. During the fifth stage experiment, the temperature ranged from 20.0 to 23.9 C and the salinity varied from 29.6 to 31.6‰. In the experiment on eleventh stage lobsters, glass immersion heaters maintained the temperature between 19 and 21 C.

In the closed seawater system, natural sea water was recirculated and filtered through dolomite and gravel, then through a fiber cartridge filter and charcoal. Temperature was controlled to match the temperature in the open system, using an immersion cooler. When necessary, distilled water was added to make up loss due to evaporation. The closed-system containers were perforated on all sides and immersed in the water. Water flowed in at one end of the water table, through all containers, exited to a second water table below, flowed through all those containers, and then to the filter, thus creating a gradient effect. For shelter each animal was provided with a hemicylinder of gray opaque PVC piping in which the animal was totally covered and able to turn around. Fifth stage lobsters were fed live brine shrimp (*Artemia salina*) nauplii and pieces of chopped mussel, *Mytilus edulis*, on alternate days. Eleventh stage lobsters were fed frozen adult brine shrimp. Feeding was done once a day in excess. Uneaten food was siphoned out prior to feeding the following day. Natural photoperiod (14L: 10D) was used for the fifth stage experiments and was maintained artificially at 14L: 10D for the eleventh stage.

Results — The second animals to molt spent a significantly longer time in the fifth stage than did the first animals to molt or the controls. There was no significant difference between the controls and the first animal to molt (Table 1). The analysis of variance showed no significant difference due to blocking. We attribute the delay in molting, which is approximately equal in the open and closed systems, to the social conditions of the pair treatment. The time spent in the fifth stage was less in the closed system than in the open system.

We continued to observe the animals in the sixth through tenth stages. The data from these stages are excluded because they are unclear due to high mortality in the paired condition and because molting became irregular. It became obvious however that the animals exhibited the same trends as those in the fifth stage, that is, paired animals showed differences in time between molts.

In the eleventh stage, a pattern of molting delay was again clearly seen. The second animal to molt spent a longer time between molts than did the first, and both showed a longer intermolt period than the control (Table 1).

TABLE 1. Mean time between molts ± standard deviation (days) in the fifth and eleventh stages for juvenile *Homarus americanus* held individually (control) and in pairs. The pair treatment is subdivided into first to molt and second to molt. Experiments were performed in both an open and a closed (recirculating) seawater system. Number of observations in parentheses.

System	Treatment	5th–6th	11th–12th
Open			
	Control	9.39±1.35(91)	35.33±4.58(9)
	1st	9.33±1.36(49)	44.86±6.82(7)
	2nd	12.35±1.82(22)	59.40±5.55(5)
Closed			
	Control	8.68±1.01(89)	30.11±2.71(9)
	1st	8.69±1.11(50)	39.70±3.71(10)
	2nd	10.78±1.40(28)	51.44±8.05(9)

The percent delay of the first and second animal to molt in the fifth and eleventh stages with respect to the control is given below.

	5th		11th	
	First	Second	First	Second
Open	0.6%	31.5%	27.0%	68.1%
Closed	0.1	24.2	31.8	70.8

In the fifth stage pairing affected mortality which in the control ranged between 2 and 7% while in the pairs ranged between 14 and 21%.

Discussion — The results presented here indicate that when held in pairs juvenile lobsters prolong the time between molts an average of 25–30%. As much as 70% delay in mean time to molt was observed for the second animal to molt in the eleventh stage when compared to the control. These observations extend Cobb's (1970) work on larval lobsters to juveniles. Stewart and Squires (1968) showed that adult lobsters delay their molt in response to crowded conditions. It thus appears that molt delay is a consistent strategy throughout all life stages of the lobster in response to communal situations. Similar responses have been seen in the land crab *Gecarcinus lateralis* (Bliss and Boyer 1964) and in the hermit crab *Diogenes pugilator* (Rossi 1971). In contrast, Lowe (1956) found no inhibition of molting rates in communally held crayfish (*Camberellus shufeldtii*).

The mechanisms for the observed delay are not clear. Douglis (1946) showed that lobsters form dominance hierarchies in captivity. Squires (1970)

and Dunham (1972) have both shown that agonistic behavior among lobsters decreases with time when they are held communally. There is thus considerable evidence that lobsters are aggressive, form dominance hierarchies, and that the dominance hierarchies help in stabilizing social structure. It may simply be that the subordinate animal, for whatever reason, molts less frequently than the dominant animal. Our observations of eleventh stage animals (Cobb and Tamm unpublished data) suggest that the dominant animal molts first and controls access to both food and shelter. Perhaps the dominant simply gets more to eat and thus molts sooner than the subordinate.

Chemical factors may also play a role in the regulation of time between molts. The intermolt period of lobsters held in the closed system were significantly shorter than those in the open system, although the temperature was kept as nearly the same as possible. Also in both the fifth and eleventh stage the standard deviation of time between molts was generally less in the closed system than in the open.

In the eleventh stage, unlike the fifth stage, the first animal of a pair to molt showed a significantly longer intermolt period than did the controls. This difference suggests that perhaps different mechanisms may be operating in the fifth stage and eleventh stage. Joan Mitchell of the Woods Hole Oceanographic Institution (personal communication) has indicated that the repertoire of agonistic behavior of juvenile lobsters is not complete until the end of the seventh stage. Thus, these observations might be interpreted as a response to one set of cues in the fifth stage and another in the eleventh. Further experimentation along these lines is planned.

Whether molt inhibition of this type is an artifact of laboratory conditions or has biological significance in the field is a moot point. It is, however, a fact that must be dealt with in aquaculture systems. Elucidation of the mechanisms of this inhibition may give us further understanding of the control of the molt cycle in lobsters, allow greater efficiency in culture and, perhaps, even allow manipulation of the molt cycle.

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A Hermaphroditic Lake Whitefish, *Coregonus clupeaformis*, from Lake Huron[1]

T. R. PORTER[2]

Lake Huron Research Unit, Fish and Wildlife Research Branch
Ontario Ministry of Natural Resources, Tehkummah, Ont.

AND S. COREY

Department of Zoology, University of Guelph, Guelph, Ont.

PORTER, T. R., AND S. COREY. 1974. A hermaphroditic lake whitefish, *Coregonus clupeaformis*, from Lake Huron. J. Fish. Res. Board Can. 31: 1944–1945.

A hermaphroditic lake whitefish, *Coregonus clupeaformis*, caught in Lake Huron is described. The specimen had a normal growth rate and had no apparent abnormal external characteristics. Both gonads were ovotestes. The ovarian and testicular portions appeared to be functional and developing normally.

PORTER, T. R., AND S. COREY. 1974. A hermaphroditic lake whitefish, *Coregonus clupeaformis*, from Lake Huron. J. Fish. Res. Board Can. 31: 1945–1945.

Les auteurs décrivent un grand corégone (*Coregonus clupeaformis*) hermaphrodite capturé dans le lac Huron. Le spécimen montre un rythme de croissance normal et n'a pas de caractéristiques externes anormales apparentes. Les deux gonades sont des ovotesticules. Les portions ovariennes et testiculaires semblent fonctionnelles et en voie de se développer normalement.

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HERMAPHRODITISM has been documented for coho salmon, *Oncorhynchus kisutch* (Crawford 1927), steelhead trout, *Salmo gairdneri* (Gibbs 1956; Ross et al. 1963). cutthroat trout, *S. clarki lewisi* (Benson 1958), chum salmon, *O. keta* (Uzmann and Hesselholt 1958), bloater, *Coregonus hoyi* (Edsall 1970), and lake whitefish, *C. clupeaformis* (Chen 1969). A second occurrence of a bisexual lake whitefish was identified and examined during routine sampling of a commercial fisherman's catch. The specimen was caught on July 16, 1970 in a 114-mm stretched mesh gillnet set on the bottom at 36.6 m in South Bay, Lake Huron, 45°36′ N, 81°55′ W. The fish was 43.4 cm in fork length, weighed 1218 g and was 8 yr old. This is a normal growth rate for lake whitefish in South Bay. The specimen had no apparent abnormal external body features.

Both gonads consisted of ovarian and testicular tissue (Fig. 1). The smaller gonad was predominantly ovarian having one testicular lobe anterior to two ovarian lobes. The larger gonad consisted of five testicular and two ovarian lobes.

Nine pieces of tissue were removed from the gonads (Fig. 1) and examined histologically. Tissue from area 1, 2, 3, 4, 6, 7, and 8 were completely testicular. In these areas early spermatogenesis appeared similar to that of a normal testis (Fig. 2). No spermatozoa were observed. Tissue from area 9 showed typical ovarian development many primary oocytes with basophilic cytoplasm. Also some mature follicles (Fig. 3) containing acidophilic cytoplasm were present. In one lobe (area 5) both maturing spermatocytes and oocytes were noted (Fig. 4). The testicular tissue predominated and virtually surrounded the ovarian tissue. In this section the testicular and ovarian tissue contained similar stages of development as seen in the corresponding lobes containing only testicular or ovarian tissues. This is similar to Edsall's (1970) description of a hermaphroditic bloater; but differs considerably from the gonads of the bisexual lake whitefish examined by Chen (1969). Chen observed that the left gonad was ovotestis and slightly smaller than the normal right testis. The ovotestis consisted of a

[1]Contribution No. 74-8 of the Fish and Wildlife Research Branch, Ontario Ministry of Natural Resources, Maple, Ont.

[2]Present address: Department of the Environment, Fisheries and Marine Service, Resource Development Branch, St. John's, Nfld. A1C 5X1

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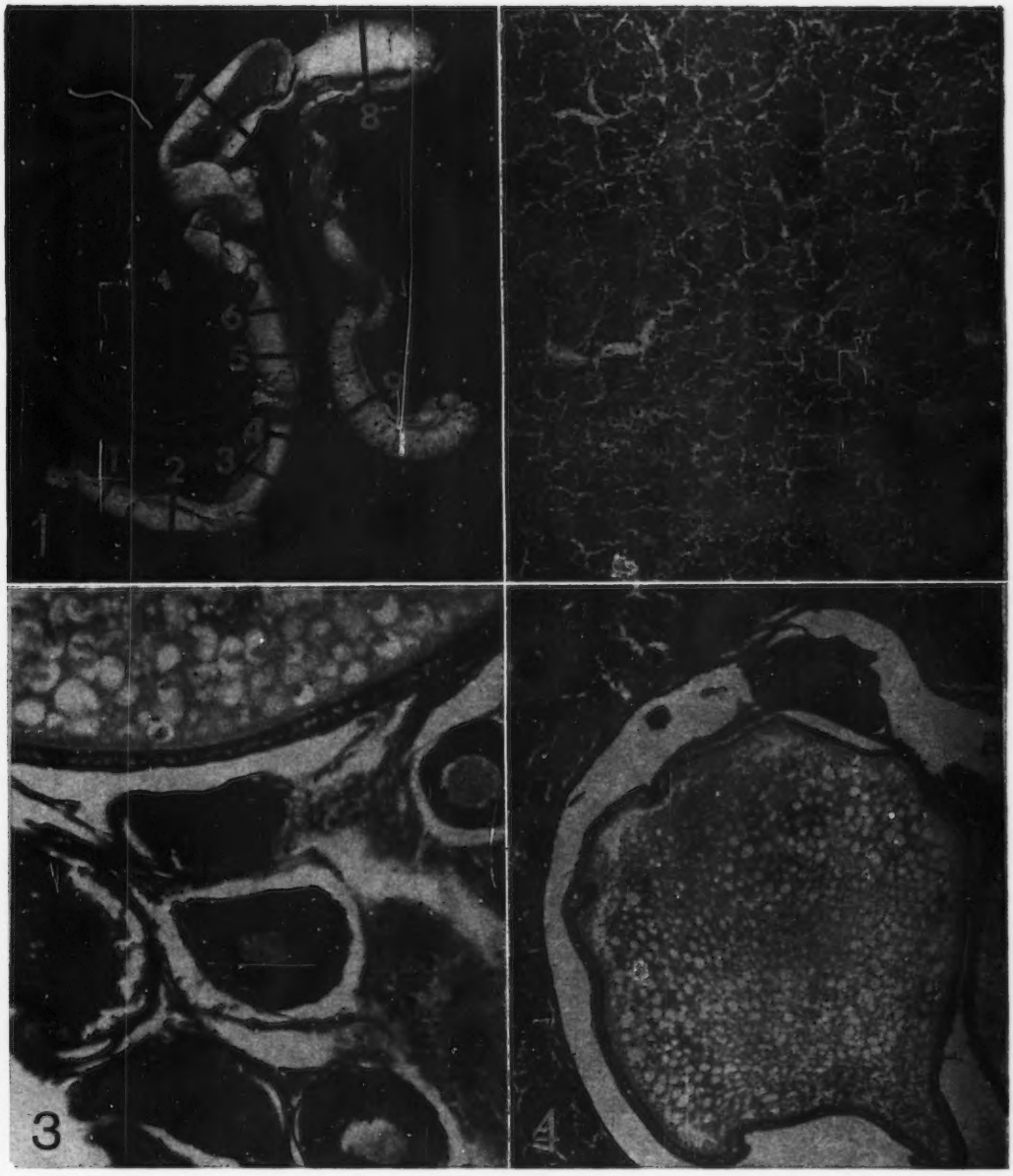

FIGURES 1–4.

FIG. 1. Gonads from a hermaphroditic lake whitefish (*Coregonus clupeaformis*) showing the areas from which histological sections were taken; FIG. 2. Section of a testicular portion of the gonad ×45, hematoxylin and eosin; FIG. 3. Section from an ovarian portion of the gonad ×100, hematoxylin and eosin; FIG. 4. Section from area 5 illustrating both testicular and ovarian tissue ×45, hematoxylin and eosin.

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small lobe (1 cm long) of ovarian tissue connected to the ventral surface of the testicular tissue by mesovarian. There was no intermingling of ovarian and testicular tissue as in the present findings.

In South Bay the majority of the male and female lake whitefish mature at 5 and 6 yr of age, respectively. Although both gonads of the hermaphrodite appeared functional there was no evidence to indicate that the fish had spawned.

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Hunger and the Capture of Grayling and Char

J. E. BRYAN[1]

Department of the Environment, Fisheries and Marine Service
Vancouver, B.C. V6E 2P1

BRYAN, J. E. 1974. Hunger and the capture of grayling and char. J. Fish. Res. Board Can. 31: 1945–1948.

Arctic grayling, *Thymallus arcticus*, and Arctic char, *Salvelinus alpinus*, were collected by angling and seining in remote areas of northern Yukon Territory. Stomach samples were observed to test the hypothesis that fish caught by angling had eaten less food than those caught by seining. The hypothesis was supported by the data for grayling. The data for char were consistent with the hypothesis, but the results were not statistically significant. There was no difference in the sex ratio of fish caught by the two methods. Angling selected larger char than did seining, but there was no evidence of size selection in grayling.

BRYAN, J. E. 1974. Hunger and the capture of grayling and char. J. Fish. Res. Board Can. 31: 1945–1948.

Nous avons capturé des ombres arctiques, *Thymallus arcticus*, et des ombles chevaliers, *Salvelinus alpinus*, à la ligne et à la senne, dans des régions éloignées du nord du Territoire du Yukon. Nous avons examiné des échantillons de contenus stomacaux afin de vérifier l'hypothèse que les poissons capturés à la ligne contiennent moins de nourriture que ceux capturés à la senne. Les données recueillies sur l'ombre arctique confirment l'hypothèse. Bien que les données sur l'omble chevalier soient conformes à l'hypothèse, les résultats ne sont pas significatifs à l'analyse statistique. La proportion des sexes est la même chez les poissons capturés par l'une ou l'autre méthode. La ligne capture des ombles chevaliers plus grands que la senne, mais il n'y a pas évidence de sélection de taille chez l'ombre arctique.

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[1]Present address: Department of Lands, Forests, and Water Resources, Pollution Control Branch, Vernon, B.C. V1T 4W5.

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Do fish strike lures because they are hungry? If all strikes at a lure are feeding responses, then there is abundant evidence that the number of strikes would increase with hunger (Holling 1966; Beukema 1968; Ware 1972). (Hunger is operationally defined as a motivational state negatively correlated with the amount of food in a stomach at a particular time). However, it is not clear that all strikes at lures are feeding responses, particularly when the lures do not mimic food. Lawler (1965) found no evidence that northern pike strike lures because they are hungry. Despite a large sample size (2252), pike caught by angling had empty stomachs no more frequently than those caught in gillnets.

Both seining and angling have been used to describe fish populations in northern Yukon Territory (Bryan et al. 1973). Although seining was the preferred method for obtaining fish samples, angling was the more expedient method in some areas because of fast or deep water. In this study, fish samples were collected by both angling and seining to determine whether the collection method biased such characteristics of the sample as size composition, sex ratio, food quantity, or food quality.

Methods — The fish were collected from remote areas of northern Yukon Territory. The grayling were captured near the headwaters of the Bell River (68° 03′N, 137°00′W) and the char were captured near the mouth of Fish Creek (69°33′N, 104°05′W) just east of Komakuk Beach Distant Early Warning Station. Because the samples were collected in such remote and sparsely populated areas, it is unlikely that the fish had been subjected to angling before their capture. The grayling were captured between 1400 and 1445 (Yukon Western Time) on August 24, 1971; the char were captured between 1300 and 1700 on August 12, 1971. A total of 0.5 man-hour was spent angling for grayling and 4 man-hours for char. The grayling were all captured in the same pool, whereas the char were collected in three different locations within a section of creek approximately 300 m long.

The lure used to collect all fish was a spinner 8 cm long with a fluorescent orange body and a bright gold blade 4 cm long. It had a treble hook 17 mm from point to point. The lure was cast with spinning gear and retrieved through the middle of the water column. After fish had been collected from a site by angling, others were collected by seining in the direction of the current. The seine measured approximately 2 × 30 m with 13-mm mesh (stretch measure) in the body and 4-mm mesh in the bunt.

About 4 h after the fish were collected, their fork lengths were measured, their gonads examined to determine sex, and their stomachs removed and preserved in 10% formalin. These samples were collected in the same order as the fish had been captured. Total volume of food was later measured, and individual organisms were identified and counted. Data on food quantity were analyzed by regression of food volume on fish length, with analyses blocked by sex, area of capture, and method of capture. Three anadromous char — distinguished by their large size (40 cm), silvery sheen, and lack of parr marks — were captured with the freshwater resident char (which had parr marks and lacked the silvery sheen). Because they had not begun active feeding in fresh water, the anadromous char were omitted from the analyses. In regression analysis of the char data, the 17 immature char were omitted when the analysis was blocked by sex. The food quality data were analyzed with chi square, but rare categories of food were omitted. Chi-square analysis assumes that the food items were eaten one at a time, and this assumption is probably warranted for the particular foods eaten.

Results — The amount of food eaten by grayling increased with their size (Table 1). Moreover, there was a difference in the form of this relationship for grayling caught by different methods:

$$\ln \mu l \text{ food angled grayling} = 6.61\,(\ln[\text{length in mm}]) - 30.02,$$

$$\ln \mu l \text{ food seined grayling} = 4.46\,(\ln[\text{length in mm}]) - 16.85.$$

Thus, grayling caught by angling usually did have less food in their stomachs than those caught by seining. A similar analysis showed that the amount of food eaten by males did not differ from that of females. Capture method had no influence on grayling size or sex ratio (Table 2).

Char caught by angling were larger but had less food in their stomachs than those caught by seining (Table 2; t-test, $P < 0.05$). However, this difference

TABLE 1. Regression of log food volume on log length of Arctic grayling. The data are blocked by method of capture. Regression tests the overall regression for both capture methods. Regression X capture methods tests slope homogeneity between capture methods. Curvature tests for additional regression on (fish length)^2. Capture method adjusted for regression uses the slope of the overall regression to test for homogeneity of intercepts between the two capture methods.

Source of variation	df	Mean square	F value	$P<$
Regression	1	1.1632	7.07	0.025
Regression × capture methods	1	0.0451	0.27	0.75
Curvature	1	0.1800	1.09	0.50
Capture methods adjusted for regression	1	1.0699	6.52	0.05
Remainder	11	0.1648		

was not as apparent in the regression analysis which takes into account the effect of fish size on the amount of food eaten. The regression was insignificant and negative when the data were blocked by capture method or sex. For data blocked by capture method, however, both the regression and capture method adjusted for regression sum of squares were large, suggesting that a larger sample size might have produced significant effects. Blocking the data by capture area produced a significant regression:

$$\ln(\mu l \text{ food}) = -1.69(\ln[\text{length in mm}]) + 15.53$$

The negative relationship indicates that large char ate proportionately less food and that char caught by angling had eaten less food partly because they were larger (Table 2). The sex ratios of char obtained using the two methods did not differ (Table 2; chi-square analysis).

The proportions of different kinds of prey eaten by fish caught by angling and seining differed for all sets of data (Table 3). The only difference which was large and consistent for all sets of data was that fish caught by angling had eaten more chironomids than those caught by seining. Less striking is the observation that fish caught by angling had eaten fewer amphipods and adult insects.

Discussion — The results are consistent with the hypothesis that both grayling and char strike lures because they are hungry. There were insufficient

TABLE 2. Size, sex ratio, and food volume of Arctic grayling and Arctic char caught by angling and seining.

Capture method	Mean length (*mm*)	SE	No. females	No. males	No. immature	Mean food volume (*ml*)	SE
			Arctic grayling				
angling	360.4	7.09	3	5	0	7.91	2.27
seining	367.2	7.09	3	5	0	14.97	2.27
			Arctic char				
angling	248.7	5.52	9	15	5	0.562	0.079
seining	231.0	4.04	19	23	12	0.740	0.058

TABLE 3. Percent of total number of food organisms eaten by Arctic grayling (G) and Arctic char (C) captured by angling (A) and by seining (S). The chi-square analysis was done on contingency tables of the food categories eaten by angled and seined fish caught in the same area (30-m stream section). All probabilities are <0.02.

Fish species	Method of capture	Area of capture	Chironomidae	Tipulidae	Simuliidae	Muscidae	Empididae	Coleoptera	Amphipoda Gammaridae	Plecoptera	Ephemeroptera	Arachnida Hydracarina	Trichoptera	Adult insects	Total no. of food organisms	Chi-square value	df	No. of fish
G	A	4	20.9	1.2	2.4	0.1	0.1	0.4		4.2	2.2	0.4	0.1	68.0	2170	114	9	8
G	S	4	13.5	1.8	0.7	0.1	0.6	0.1		2.8	1.7	0.2	1.3	77.2	2667			8
C	A	1	54.2	0.9	0.1	0.1			23.0	6.2	0.5	0.4		14.4	1055	81	6	12
C	S	1	41.3	2.6	0.1	0.1			25.0	13.4	1.2	1.1		15.2	2213			25
C	A	2	54.1						29.2	3.1	0.1	0.1		13.4	1147	14	5	11
C	S	2	48.7	0.3	0.1	0.4			31.8	0.3		0.8		13.9	1350			10
C	A	3	60.1	1.0		0.4			21.4	4.8	0.1	0.4		11.5	668	211	7	6
C	S	3	31.0	1.8	0.2	0.1			43.8	7.8	0.6	0.4	0.6	13.7	2787			19

data for char to provide a thoroughly convincing test of the hypothesis. The differences in amount of food may have been slight because all of the char were relatively hungry, and none of them were sufficiently satiated to ignore potential food. The results do not imply that hunger is the only condition which causes fish to strike lures — hunger merely increases the likelihood that they will strike lures.

Feeding rate decreases as fish become satiated (Beukema 1968; Ware 1972). Consequently, one would expect angling to select for hungry fish if it were clear that the lure used mimics natural food objects and if a strike were always a feeding response. With the spinner I used to catch fish, there was no a priori reason to assume that the fish would regard the lure as food. A strike might have reflected aggression or displacement activity. The results, however, suggested that grayling and char did regard the lure as food and that a strike was a feeding response. Angling may have failed to select for hungry pike (Lawler 1965) because pike were motivated by aggression more than by hunger. Westman (1961) argues that hunger is not all that motivates fish to strike lures. It is also possible that the observations on pike (Lawler 1965) resulted because of digestion while pike were in the gillnets or because hungry pike spend more time searching for food.

It may be important that the fish studied probably had no previous experience with lures. Negative reinforcement from previous experience with similar lures would have prevented some fish from striking, thus increasing the variability in results. Beukema (1970) found that just one experience with angling induced subsequent avoidance in carp which persisted for at least 1 yr.

Angling selected for larger char, whereas there was no size selection of grayling. The char population included a much larger range of sizes than did the grayling population. Angling may have selected large char because they were hungrier. Alternatively, the small char may have had difficulty getting the hooks into their mouths, or a social hierarchy may have enabled larger char to strike the lure first. The grayling, all being large, were not differentially vulnerable to angling because of differences in size.

The differences in kind of food selected by fish caught with the two methods were probably spurious. If the lure mimicked a kind of food eaten naturally, then one would expect to find that fish caught by angling had eaten a larger proportion of the mimicked food than had those caught by seining. This would be expected because individuals specialize on particular foods (Bryan and Larkin 1972). Other data on grayling support this contention. Grayling caught with dry flies had eaten more of surface food types than those caught with wet flies (G. L. Vascotto personal communication). As there is no obvious similarity between the spinner I used and the food types eaten, one would expect to find no difference in the kinds of food eaten. The finding that there were differences in food of angled and seined fish probably reflects that there are usually large differences in the kinds of food eaten by different individuals (Bryan and Larkin 1972).

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Toxicity of Copper at Two Temperatures and Three Salinities to the American Lobster (*Homarus americanus*)

D. W. McLeese

Department of the Environment, Fisheries and Marine Service
Biological Station, St. Andrews, N.B. E0G 2X0

McLeese, D. W. 1974. Toxicity of copper at two temperatures and three salinities to the American lobster (*Homarus americanus*). J. Fish. Res. Board Can. 31: 1949–1952.

The time to 50% mortality (LT50) for lobsters at a particular lethal concentration of copper is longer at 5 than at 13 C but is not affected by salinities of 20–30‰. The lethal threshold of copper for lobsters at temperatures of 5 and 13 C and at salinities of 20 to 30‰ is close to 56 μg/liter.

McLeese, D. W. 1974. Toxicity of copper at two temperatures and three salinities to the American lobster (*Homarus americanus*). J. Fish. Res. Board Can. 31: 1949–1952.

Le temps requis pour des mortalités de 50% (TL50) de homards maintenus à une concentration létale particulière de cuivre est plus long à 5 qu'à 13 C, mais n'est pas affecté par des salinités de 20 à 30‰. Le seuil létal de cuivre pour des homards à des températures de 5 et 13 C et des salinités de 20 à 30‰ se rapproche de 56 μg/litre.

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In static tests with lobsters exposed to sheets of metal, Wilder (1952) found that copper, monel metal, zinc, and lead were toxic in that order. When copper, zinc, and lead were retested in a similar arrangement, with a slow exchange of water, copper and zinc were again shown to be toxic, although more slowly, presumably because of lower concentrations (D. W. McLeese, unpublished data). No information on metal concentration was obtained in either experiment.

Biesinger and Christensen (1972) found that only 3 of 21 metals tested, cobalt, mercury, and cadmium, were more toxic than copper to *Daphnia magna*. Other authors have also indicated that copper is among the more toxic metals to aquatic animals or at least that copper is more toxic than zinc (e.g. Portmann 1968; Wiseley and Blick 1967; Pickering and Henderson 1966; and Sprague 1964). There are indications that decrease in temperature reduces the toxicity of metals (Portmann 1968; Sprague 1964).

Toxicity of copper to lobsters was studied to provide a measure of lethal concentrations and to observe whether its toxicity is affected by temperature and salinity. Assuming copper is one of the most toxic metals, the information will provide a rough indication of the sensitivity of lobsters to metals in general. More directly, the information will provide a basis for assessing safe levels of copper for lobsters in their natural environment and during their commercial storage.

Materials and methods — Lobsters, each weighing about 450 g, were tested at temperatures close to their natural acclimation temperature, 13 C in summer and early fall and 4 C in early winter. The animals were not fed for at least 5 days before nor during testing.

Groups of five lobsters were tested in running water at copper concentrations from 1 to 1000 μg/liter. The test tanks contained 60 liters with a seawater flow of 0.5 liters/min, providing 50% water replacement in 1.3 h and 90% replacement in 6 h (Sprague 1969). Constant head Mariotte bottles delivered copper sulphate solution at the rate of 0.7 ml/min. Copper concentrations in the Mariotte bottles were such that the toxicant flow added to the seawater flow resulted in the desired copper concentrations in the test tanks. The solutions were acidified to pH 1.5–2.0 to prevent copper from plating out on the walls of the containers.

Dechlorinated fresh water was mixed with sea water in the constant level header tanks to provide reduced salinities of 20 and 25‰.

Water temperature, salinity, dissolved oxygen, pH, and flow rates of water and toxicant for each tank were measured daily (Table 1). Copper concentrations were measured, usually once a day, by the sodium diethyldithiocarbamate method (Sandell 1959).

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TABLE 1. Summary of experimental factors in toxicity tests.

Factor	Stated level	Range of measurements		
Temp	5 C	4.5–6.0 C		
	13 C	12.0–14.0 C		
Salinity	30‰	30.0–31.0‰		
	25‰	23.3–26.3‰		
	20‰	18.3–21.5‰		
Dissolved oxygen at	5 C — 11 ppm	9–11 ppm		
at	13 C — 9 ppm	7–10 ppm		
pH	8.0	7.8–8.1		
Water flow/tank	0.5 l/min	0.48–0.52 l/min		
Toxicant flow/tank	0.7 ml/min	0.6–0.75 ml/min		
		No.	*Mean*	*Range*
Copper concn	1000 µg/l	4	890	840–1000
	560	8	470	360–540
	100	28	104	78–108
	56	28	54	38–78
	32	25	35	25–54
	10	7	11	5–13
	1	6	3	2–5

Lobsters were considered to be dead when no movement of any appendage could be detected.

Times to 50% mortality (LT50s) and their 95% confidence limits were estimated by probit analysis (Litchfield 1949). Pairs of LT50s were compared using the reaction ratio method (Litchfield 1949) to test for differences between replicates and to test for temperature and salinity effects. Lethal thresholds were estimated from plots of LT50s in relation to copper concentration.

Results — Times to 50% mortality were obtained at copper concentrations down to 100 µg/liter, at all temperatures and salinities tested (Table 2). An exception was 1 of 11 tests at 100 µg/liter where all lobsters appeared normal at 436 h. In 22 of 24 tests at concentrations of 56 µg/liter and lower, 50% mortality did not occur during exposures of 311–665 h. Both exceptions were at 56 µg/liter, one at 13 C and 30‰ salinity, the other at 5 C and 20‰.

Underlined LT50s (Table 2) are significantly shorter than those from replicate tests ($P = 0.05$). The LT50s at the two temperatures within a copper concentration and salinity, were significantly longer at 5 than at 13 C in 29 of 34 comparisons. Of the remainder, three pairs showed no difference with temperature and two pairs had shorter LT50s at 5 C. Three of the latter five comparisons involved LT50s that were less than their replicates. It is concluded that a particular lethal concentration of copper is more rapidly lethal to lobsters at 13 than at 5 C.

Comparison of LT50s at 30‰ salinity with those at 25 and 20‰, within a given temperature and copper concentration, showed no difference with salinity in 20 of 38 comparisons. Of the remainder those at 30‰ were greater than those at lower salinities in 4 comparisons and were less in 14 comparisons. Of the latter 18 showing a difference, 16 involved LT50s that were less than their replicate values. It is concluded that salinity over the range 20–30‰ does not affect the toxicity of copper to lobsters.

Regardless of the temperature effect, the lethal threshold for copper at 5 and 13 C at salinities of 20–30‰ lies between 100 and 56 µg/liter, presumably close to 56 µg/liter (Table 2).

Discussion — The average time to death of lobsters exposed to a sheet of copper in a static test at about 12 C was 14 h (Wilder 1952). To kill lobsters at approximately 14 h, the copper concentration probably would have been about 10,000 µg/liter (Table 2). In a similar arrangement but with a water exchange of 1 liter/min into 224 liters, the LT50 at 5 C was 75 h (71–79) (McLeese unpublished data). The copper concentration during that test would probably have been about 560 µg/liter (Table 2).

It is possible that toxicity of metals to marine and estuarine animals would change with variation in environmental factors such as temperature, salinity, and pH. For freshwater organisms, it has been shown that hardness and pH alter the toxicity of most heavy metals (Skidmore 1964; Malacea and Gruia 1964) and that humic acid increases the incipient lethal level of copper to juvenile Atlantic salmon (*Salmo salar*) (Zitko et al. 1973). The tolerance of the brown shrimp (*Crangon crangon*) to copper was increased by a factor of 5 following a reduction in temperature from 22 to 5 C (Portmann 1968).

For lobsters exposed to a particular lethal concentration of copper, the LT50s increased by a factor of about 2 with a temperature change from 13 to 5 C, but the lethal threshold (incipient lethal level) was not affected.

Salinity over the range 20–30‰ did not affect the toxicity of copper to lobsters. These salinities are above the range of lethal low salinities (McLeese 1956). Because of the buffering capacity of sea water only minor changes in pH would be expected and it remained close to 8 in these tests (Table 1).

Aquatic animals with similar sensitivity to copper as the lobster include the freshwater amphipod, *Gammarus pseudolimnaeus* (Arthur and Leonard 1970), the pink shrimp, *Pandalus montagui* (Portmann 1968), the waterflea, *Daphnia magna* (Biesinger and Christensen 1972), the mussel, *Mytilus edulis*

TABLE 2. Toxicity of copper to American lobsters (*Homarus americanus*) at two temperatures, each at three salinities. Underlined LT50s are significantly shorter than those from replicate tests ($P = 0.05$).

Temp (C)	Test concn (μg/l)	30‰ salinity LT50 (h)	30‰ salinity 95% CL (h)	25‰ salinity LT50 (h)	25‰ salinity 95% CL (h)	20‰ salinity LT50 (h)	20‰ salinity 95% CL (h)
13[a]	1000	22	17–28	–		–	
		33	30–36	–		–	
		26	20–35	–		–	
	560	36	31–42	45	37–54	24	18–32
		52	45–59	52	41–66	44	38–51
			–	36	33–40	40	35–45
	100	72	64–80	120	83–174	128	99–173
		140	111–170	320	262–390	0/5 dead at 436 h	
	80	105	92–120		–		–
	56	2/5 dead at 312 h		1/5 dead at 311 h		0/5 dead at 311 h	
		285	196–413	1/5 dead at 436 h		0/5 dead at 436 h	
	32	0/5 dead at 312 h		1/5 dead at 311 h		0/5 dead at 311 h	
		0/5 dead at 665 h		1/5 dead at 436 h		0/5 dead at 436 h	
	10	1/5 dead at 453 h			–		–
		0/5 dead at 521 h			–		–
13 C	1	1/5 dead at 453 h			–		–
	0 (control)	0/5 dead at 449 h			–	1/5 dead at 311 h	
			–		–	0/5 dead at 436 h	
5 C	1000	52	46–59		–		–
		58	48–69		–		–
	560	70	58–84	72	60–90	78	62–98
		72	65–80	66	56–77	80	63–101
	100	172	144–207		–	310	286–336
		270	211–345	275	240–310	330	295–370
	56	1/5 dead at 328 h		1/5 dead at 450 h		210	113–388
		2/5 dead at 500 h			–	2/5 dead at 427 h	
	10	1/5 dead at 328 h		0/5 dead at 450 h		0/5 dead at 560 h	
		0/5 dead at 500 h			–		–
	0 (control)	0/5 dead at 500 h		0/5 dead at 450 h		0/5 dead at 560 h	

[a] A static test at 13 C, 30‰ salinity, and 10,000 μg/liter gave an LT50 of 14 h.

(Adema et al. 1972), the fathead minnow, *Pimephales promelas* (Mount 1968; Mount and Stephan 1969) and young Atlantic salmon, *S. salar* (Sprague 1964; Zitko et al. 1973). Some with considerably lower sensitivity are the brown shrimp, *C. crangon*, with a 48-h LC50 of about 30,000 µg/liter, the shore crab, *Carcinus maenas*, with a 48-h LC50 of about 100,000 µg/liter (Portmann 1968) and the oyster with a 96-h LC50 of 1900 µg/liter (Fujiya 1960). For comparison, approximations of 48-h LC50s for lobsters, as interpolated from Table 2, are about 560 µg/liter at 13 C and 1000 µg/liter at 5 C. The 96-h LC50 is slightly more than 100 µg/liter at 13 C and slightly less than 560 µg/liter at 5 C.

To more fully assess the potential hazard of copper to lobsters, study of the sensitivity of lobster larvae to copper, under a variety of environmental conditions, is also required.

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Changes in Blood Chemistry of Juvenile Steelhead Trout, *Salmo gairdneri*, Following Sublethal Exposure to Nitrogen Supersaturation

T. W. NEWCOMB

Northwest Fisheries Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, Wash. 98112, USA

NEWCOMB, T. W. 1974. Changes in blood chemistry of juvenile steelhead trout, *Salmo gairdneri*, following sublethal exposure to nitrogen supersaturation. J. Fish. Res. Board Can. 31: 1953–1957.

Groups of juvenile steelhead trout (*Salmo gairdneri*) were exposed for 35 days to various (103, 105, 110, and 116%) sublethal nitrogen plus argon saturations. Pooled serum samples were analyzed for Ca, Na, PO_4, K, Cl, albumin, total protein, cholesterol, alkaline phosphatase, glucose, urea, uric acid, total bilirubin, lactate dehydrogenase, and serum glutamic oxalacetic transaminase. An increase in serum potassium and phosphate, and a decline in serum albumin, calcium, cholesterol, total protein and alkaline phosphatase were noted in steelhead exposed to 116% nitrogen (N_2 + Ar) saturation (total atmospheric gas saturation 110%). No major changes in blood chemistry were observed at nitrogen saturations of 110% or less.

NEWCOMB, T. W. 1974. Changes in blood chemistry of juvenile steelhead trout, *Salmo gairdneri*, following sublethal exposure to nitrogen supersaturation. J. Fish. Res. Board Can. 31: 1953–1957.

Des groupes de jeunes truites steelhead (*Salmo gairdneri*) ont été exposées durant 35 jours à diverses saturations sublétales (103, 105, 110 et 116%) d'azote plus argon. Sur des échantillons de sérum combinés, nous avons déterminé Ca, Na, PO_4, K, Cl, albumine, protéines totales, cholestérol, phosphatase alcaline, glucose, urée, acide urique, bilirubine totale, lactate déshydrogénase et glutamique oxalacétique transaminase sérique. Nous avons observé une augmentation du potassium et du phosphate sériques, et une diminution, dans le sérum, de l'albumine, calcium, cholestérol, protéines et phosphatase alcaline chez des truites exposées à une saturation d'azote (N_2+Ar) de 116% (110% de saturation des gaz atmosphériques totaux). Nous n'avons pas observé de changements majeurs dans la chimie du sang à des saturations d'azote de 110% ou moins.

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ONE major water pollution problem of the Columbia and Snake rivers is atmospheric gas supersaturation which produces gas-bubble disease in fish.

Gas emboli form in the fish's supersaturated blood in some as yet undefined manner (B. G. D'Aoust, Hyperbaric Laboratory, Virginia Mason Research Center, Seattle, Wash., personal communication). Gas emboli may then lodge at a variety of sites throughout the fish's body. These emboli can then limit delivery of oxygen, and removal of carbon dioxide and toxic metabolites from the effected tissues and organs. Persistence of this physiological condition will lead to the ultimate death of the fish.

A number of mortality studies with nitrogen gas supersaturation have been made on juvenile salmonids, but little work has been published on the sublethal effects (Dawley and Ebel 1974). Sublethal effects from gas supersaturation may cause alterations in the fish's blood chemistry which can be used as tools to determine the degree of stress. These blood chemical measurements may help pinpoint potential long-range problems encountered by the juvenile salmonid such as ingestion and assimilation of food, predator avoidance, locomotion, homing behavior, fecundity, and the quantity and quality of subsequent fertilized eggs and fry. In addition, measuring these blood chemistry characteristics may allow in situ evaluation of nitrogen gas supersaturation stress response both singly and in combination with other normally occurring stressors (e.g. passage through turbines) which occur to the juvenile salmonid on its down-stream migration.

This blood chemistry experiment represents an extension of a bioassay by Dawley and Ebel (1974)

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which was designed to provide a variety of lethal and sublethal biological criteria which would help form the basis for an EPA provisional water quality standard. The primary goal was the establishment of a series of LE_{50} values. Secondary goals were changes in voluntary swimming performance and blood chemistry characteristics which might give some estimate of sublethal stress response to gas-bubble disease.

Materials and methods — The fish used in these experiments were steelhead trout, *Salmo gairdneri*, provided by the Washington State Department of Game Hatchery at Aberdeen, Wash. They weighed 20.5 ± 6.3 g and measured 132 ± 17 mm in fork length at the completion of these tests. Fish were acclimated to water temperatures of 15.0 ± 0.5 C (5 C/wk with 2 wk at 15 C) in acclimation tanks described by Ebel et al. (1971). Fish were fed once each day a maintenance ration of Oregon Moist Pellets.

Fish were exposed to various nitrogen supersaturation levels in shallow rectangular tanks (Dawley and Ebel 1974) with water 23 cm deep from the same source as used in the acclimation tanks. Tests were performed at 15.0 ± 0.5 C at nitrogen gas (N_2 + Ar) saturations of 103.5, 105.4, 110.1, and 116.0 ± 2.0% as calculated from Weiss (1970). These nitrogen saturations corresponded to total gas pressure (TGP) saturations (N_2 + Ar + O_2) of 99.9, 102.0, 105.9, and 110.2%. Mean oxygen saturations ranged between 87.5 and 90.5%. Fish were continued on the same maintenance ration of Oregon Moist Pellets during these tests.

Sixty fish were taken concurrently from the acclimation tanks and placed in each of three experimental and three control tanks (102.0 vs. 99.9; 105.9 vs. 99.9; and 110.2 vs. 99.9% TGP). Tests at each saturation level were continued until 35 days had elapsed (the 35-day test period was chosen to parallel the average exposure time of seaward migrant steelhead passing downstream through the nitrogen supersaturated waters of the lower Snake and Columbia rivers). At this time, each fish was given an individual voluntary swimming performance test (2–5 min) in an inclined vee trough against a water current of 1.28 m/s (15.0 ± 0.5 C) (Schiewe 1974). A variety of experimental limitations dictated that these fish must be used in the swimming performance test before being used in the present blood chemistry work. Therefore, to minimize short-term blood chemistry changes due to the swimming challenge, each experimental and control group was placed in a control tank for 24 h before blood sampling (Grant and Mehrle 1973).

Fish were then individually anesthetized with neutralized M.S. 222 (Wedemeyer 1970a) in a concentration sufficient to reach plane three anesthesia in about five min. Blood was collected from the severed caudal peduncle and processed after Miles and Smith (1968) except that plain hematocrit tubes were used to obtain serum. These fish were then weighed and examined for signs of gas-bubble disease. Individual serum samples were pooled within each experimental or control group and immediately sealed and frozen at 0 C until chemical analysis was performed. Pooling was necessary to make up the minimum sample volume needed for the following analysis.

A Technicon SMA 12–60 (Sequential Multiple Analyzer) was used to determine serum calcium, phosphate, glucose, urea, uric acid, cholesterol, total protein, total biliruben, alkaline phosphatase (AP-ase), lactate dehydrogenase (LDH), and glutamic oxalacetic transaminase (SGOT). All samples were run sequentially with a reference synthetic serum (General Diagnostic's Calibrate Automated "Lock-in"), while three control synthetic sera (General Diagnostic's Calibrate) were used to calibrate the output of each of the twelve channels. Measurement error was less than 1.0% with the exception of cholesterol and LDH which were 1.2%. Serum calcium was determined by the method of Kesler and Wolfman (1964) as modified by Technicon. Inorganic phosphate was detected by a Technicon modification of the method of Fiske and Subbarow (1925). Glucose was detected by a modification of the procedures of Brown (1961), and Bittner and McCleary (1963). Urea nitrogen was determined by a modification of the method of Marsh et al. (1965). Uric acid is measured by a Technicon modification of the cupric-neocuproine procedure originally described by Bittner et al. (1963). A Technicon modification of the Lieberman-Burchard reagent was used in the direct determination of serum cholesterol. Total protein was quantitated by the Technicon modification of the biuret reaction. The HABA anionic dye [(2-(4′-hydroxyazobenzene) benzoic acid)] used in the Technicon SMA 12/60 was unusable for salmonid albumin, and a manual procedure using the bromcresol green method of Doumas et al. (1971) was substituted in these experiments. The method for the estimation of total bilirubin is based on a Technicon modification of the procedure of Jandrassik and Grof (1938). Alkaline phosphatase was determined by a modification of the King–Armstrong procedure developed by Marsh et al. (1959). LDH was measured by a method based on the procedure of Hochella and Weinhouse (1965). Glutamic oxalacetic transaminase was quantitated after the procedure of Morgenstern et al. (1966).

Serum sodium and potassium were measured on a Corning Model 170 digital flame photometer, while serum chloride was determined by the method of Schales and Schales (1941). Correlation coefficients were calculated for all of the above blood characteristics along with oxygen, nitrogen, and TGP saturations, and mean wet weight and fork length.

Water analysis for nitrogen and oxygen were performed daily in each test tank using the methods of Dawley and Ebel (1974). Weekly measurements of total alkalinity (16–20 ppm), total hardness (10–21 ppm), carbon dioxide (1.2–2.0 ppm), chlorine + chloramine (<0.02 ppm) and pH (6.8–7.3) were made according to Tarus et al. (1971). Calcium (6.4–6.7 ppm) and potassium (0.3–1.0 ppm) were determined monthly by flame spectrophometer.

Results — Alteration from control mean values ($>3\sigma$) were observed in 7 of 16 blood characteristics in juvenile steelhead exposed to 116.0% nitrogen saturation, while meaningful changes in these 16 substances were not noted at lower saturations (Fig. 1). An increase in concentrations of potassium and phosphate was noted at 116.0% nitrogen saturation, while a decrease was noted in albumin, calcium, cholesterol, AP-ase, and total protein. Decrease in serum calcium was positively correlated with decreases in cholesterol ($r = .86$; $P<0.05$) and albumin ($r = .95$; $P<0.01$), and negatively correlated with an increase in potassium ($r=-.77$; $P<0.05$).

In addition to potassium, a decrease in albumin was negatively correlated with an increase in phosphate ($r=-.82$; $P<0.05$), and positively correlated with decreases in cholesterol ($r=.97$; $P<0.01$) and total protein ($r=.90$; $P<0.01$).

No correlation ($P<0.5$) could be drawn between oxygen, nitrogen, or TGP saturation and mean weight or length.

A 46% incidence of external gas-bubble disease signs was noted in steelhead exposed to 116.0% saturation, while no external signs were observed at lower saturations. These signs were largely in the form of lateral line bubbles with a lower incidence of blebs appearing in the dorsal and caudal fins. No mortalities occurred during these tests.

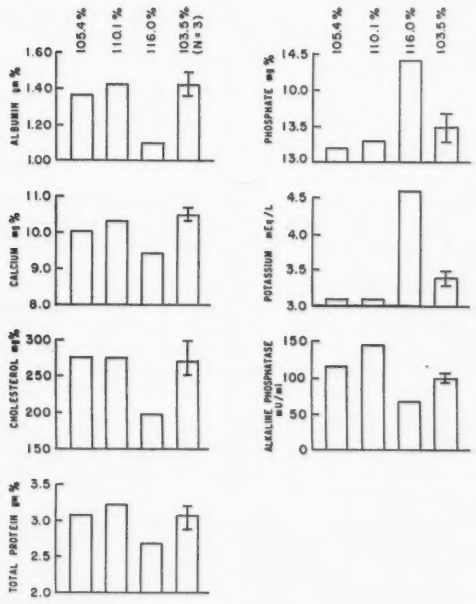

FIG. 1. Major serum chemistry parameters with increasing N_2 + Ar saturations. Control (103.5% N_2 + Ar) values are mean and range.

Discussion — It is important to distinguish between blood chemistry changes due to chronic as opposed to acute (short-term) stressors (Wedemeyer 1970b). Much of the literature on salmonid blood chemistry is concerned only with acute stressors; those applied over 1h–1 wk (Cardwell et al. 1970; Miles and Smith 1968; and Wedemeyer 1970a and b, 1971, and 1972). Because no mortality occurred over the 35-day test at 116% nitrogen (110% TGP), I considered only chronic stressor responses in this discussion. The chronic stress noted at 110% TGP is particularly significant because 110% TGP saturation is the level presently suggested by EPA as a provisional water quality standard (R. L. Rulifson, Environmental Protection Agency, Seattle, Wash., personal communication).

Two mechanisms have been invoked in an attempt to explain gas-bubble disease. These mechanisms are gas embolism of the heart, gills, or other vital portions of the blood system (Marsh and Gorham 1905; Dawley and Ebel 1974), and starvation due to blebs in the lining of the mouth preventing ingestion of food (Dawley and Ebel 1974). A 50% reduction in serum glucose was indicated by Robertson et al. (1963), when rainbow trout were starved. Similarly, albumin (Booke 1964) and total protein (Phillips et al. 1960) decreased in brook trout, *Salvelinus fontinalis*.

Emboli may be envisioned to block arteries and veins in a variety of critical tissues and organs, which would result in local or systemic hypoxia, respiratory acidosis and necrosis from the buildup of toxic wastes. The gills, brain, heart, and kidney are four such critical sites. Short-term hypoxia was shown to cause severe hemoconcentration, a doubling of serum phosphate, a 60% increase in serum glucose, and an uncompensated respiratory acidosis in Atlantic menhaden, *Brevoortia tyrannus* (Hall 1928). Extreme hypoxia in the brown bullhead, *Ictalurus nebulosus*, and the American eel, *Anguilla rostrata*, in fresh water, resulted in severe albuminemia (Bieter 1931). Hunn (1969) found that rainbow trout subjected to hypoxic stress showed a doubling of plasma phosphate.

Juvenile steelhead stressed for 35 days at 116.0% nitrogen saturation show no significant decrease in mean weight or length when compared to other test groups. These data would tend to support the hypoxia argument at the expense of the starvation proposition. Hypoxia should show an increase in serum cholesterol (Wedemeyer 1970a), but the present data suggest that cholesterol in fact declines

in steelhead exposed to 116.0% nitrogen saturation. While steelhead exposed to 116.0% nitrogen saturation alter their serum concentrations of phosphate (Hunn 1969) and calcium (Wedemeyer 1971) in a manner consistent with the hypoxia thesis, comparable data for the starvation interpretation is missing. In reciprocal fashion decreases in albumin and total protein are known (Phillips et al. 1960) following a period of starvation, but comparable data for chronic hypoxia is needed. Steelhead serum potassium and AP-ase levels need to be determined for both chronic hypoxia and starvation.

Further work will be necessary to ascertain whether these signs reflect hypoxia and tissue necrosis, starvation, or some generalized stressor response.

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Indirect Fluorescent Antibody Technique for Detection of RM Bacterium of Rainbow Trout (*Salmo gairdneri*)

G. R. JOHNSON, G. WOBESER

Department of Veterinary Pathology
Western College of Veterinary Medicine, University of Saskatchewan
Saskatoon, Sask. S7N 0W0

AND B. T. ROUSE

Department of Veterinary Microbiology
Western College of Veterinary Medicine, University of Saskatchewan
Saskatoon, Sask.

JOHNSON, G. R., G. WOBESER, AND B. T. ROUSE. 1974. Indirect fluorescent antibody technique for detection of RM bacterium of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 31: 1957–1959.

An indirect fluorescent antibody technique was evaluated as a method for the rapid detection of RM bacterium of rainbow trout (*Salmo gairdneri*). Antibody produced by inoculating rabbits with washed RM bacterium antigen was combined with commercially prepared fluorescein-labelled antiglobulin. The technique demonstrated strong specificity for the RM bacterium in washed and broth cultures, and in kidney smears from experimentally infected rainbow trout.

JOHNSON, G. R., G. WOBESER, AND B. T. ROUSE. 1974. Indirect fluorescent antibody technique for detection of RM bacterium of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 31: 1957–1959.

Nous évaluons une technique indirecte à anticorps fluorescents comme méthode de détection rapide de la bactérie RM de la truite arc-en-ciel (*Salmo gairdneri*). L'anticorps obtenu par inoculation de l'antigène lavé de la bactérie RM dans des lapins est combiné avec une préparation commerciale d'antiglobuline marquée à la fluorescine. La technique démontre une haute spécificité pour la bactérie RM dans des cultures lavées et des bouillons, et dans des frottis de rein de truites arc-en-ciel infectées expérimentalement.

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ENTERIC Redmouth (ERM)[1] is a disease of rainbow trout (*Salmo gairdneri*) caused by the "RM bacterium"[2] characterized by Ross et al. (1966). Although ERM has been reported only once in Canada (Wobeser 1973), rainbow trout imported into Canada may originate in areas in which the disease has been reported or is considered enzootic. Fluorescent antibody techniques (FAT) provide a rapid specific method of diagnosis which has been used for the identification of other fish pathogens (Klontz and Anderson 1968; Lewis and Allison 1971), and are widely used in medical and veterinary diagnostic microbiology.

An indirect FAT for the detection of the RM bacterium is described.

[1,2]Name of disease and etiologic agent are those adopted by the Fish Health Section, American Fisheries Society at a Workshop on Technical Procedures and Professional Standards, Denver, Colo., August 12–15, 1974.

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Materials and methods — A single colony of RM bacterium isolated from an infected rainbow trout during the outbreak reported by Wobeser (1973) was used as the test organism. The organism was inoculated into 1-liter bottles containing 500 ml of trypticase soy broth which were incubated at 22 C on a shaker (88 oscillations/min) for 48 h. The cultures were centrifuged at 5650 *g* for 5 min and the supernatant was decanted. Cultures were washed once in several volumes of isotonic sterile saline, centrifuged, and the supernatant was discarded. One of the cultures, (hereafter referred to as antigen), was suspended in three volumes of 0.5% formalin saline at 22 C for 24 h, washed 3 times in several volumes of sterile isotonic saline, and samples were plated on trypticase soy agar plates to confirm sterility.

The number of organisms in a second culture was determined by serial dilution and plate count. A suspension containing 10^9 cells/ml corresponded in visual density to a McFarlane standard #1, which was subsequently used as a standard for the dilution of the antigen.

Four New Zealand White rabbits were used to produce RM bacterium antibody. A 10-ml blood sample was obtained from each rabbit prior to the first inoculation. One rabbit (Number 1) was inoculated intravenously with antigen at 4-day intervals. The amounts of antigen administered were 0.5 ml, 1.0 ml, 2.0 ml, and 3.0 ml. The rabbit was test bled on day 18 and 3.0 ml of antigen was administered on day 21. The rabbit was exsanguinated on day 27.

Three rabbits (Number 3–5) were each injected subcutaneously with 1.0 ml of antigen on days 0, 4, 12, 18, and 25; test bled on day 32; and exsanguinated on day 42. Serum was harvested and stored in 2-ml aliquots at –23 C.

The antibody titer of the pre- and postinoculation sera was determined by tube agglutination using serial doubling dilutions from 1/10 to 1/1280. Each series included a saline control. An equal volume of antigen was added to each dilution and the tubes were incubated at 37 C for 24 h.

The following technique was used for all fluorescent antibody tests: 0.25 ml of the test bacteria was placed within 1-cm squares etched on glass slides, dried, fixed in methanol at 20 C for 15 min, and washed in running tap water for 1 h. Serum harvested from the rabbits (anti-RM serum) was flooded in the squares and the slides were incubated in a moist chamber for 30 min, and then washed with three changes of phosphate buffered saline (PBS), pH 7.2. Labelled antiglobulin[3] was added and the slides were incubated in a moist chamber for 30 min, washed with three changes of PBS, and coverslips were mounted with 1% glycerol saline.

Fluorescent microscopy was performed with a Carl Zeiss Ultraphot III using incident light from an Osram HBO 200 lamp with BG 3 exciter filter in combination with a permanent BG 38 filter and 50/44 barrier filter. These filters gave a peak excitation intensity of 320–400 nm.

The optimal dilutions of anti-RM sera and labelled antiglobulin determined by titration were tested for cross reactivity with *Aeromonas liquefaciens*, *A. salmonicida*, *Vibrio anguillarum*,[4] *Escherichia coli*, and Salmonella Group B+[5].

A preliminary trial was performed to determine the usefulness of this technique for the detection of RM bacterium in fresh fish tissue. Rainbow trout inoculated intraperitoneally with 0.1 ml of 48-h broth culture of RM bacterium were killed when moribund and kidney impression smears were fixed and prepared by the technique described above. Impression smears from kidney of uninfected fish from the same group, maintained in a separate container, were used as a control.

Results — Test bleedings of the rabbits during the inoculation schedule and at the time of exsanguination demonstrated increasing agglutination titers for RM bacterium. Intravenous inoculation produced a higher titer (1/640) than did the subcutaneous route (1/320). Preinoculation sera, saline controls, and labelled antiglobulin did not agglutinate the antigen.

Fluorescence was subjectively rated to establish optimal specific fluorescence with minimal background fluorescence. Anti-RM sera from rabbits 1 and 5 at dilutions of 1/160 and 1/80, respectively, (corresponding agglutination titers 1/640, 1/320), were used to titrate the labelled antiglobulin. The optimal titers of labelled antiglobulin (rabbit 1: 1/40; rabbit 5: 1/30) were used to titrate the anti-RM sera. The optimal dilutions of anti-RM sera were 1/320 and 1/160 for rabbits 1 and 5, respectively.

There was no specific fluorescence when preinoculation sera or saline controls were used in place of anti-RM sera; however, background fluorescence with preinoculation sera was similar to that seen with anti-RM sera.

The FAT demonstrated no cross reactions with washed or broth cultures of the other bacteria tested. A pale green haze over concentrations of *E. coli* and *V. anguillarum* was dull, diffuse, and easily distinguishable from the brilliant halo around the RM bacterium.

The organism stained specifically on kidney smears from experimentally infected fish; however, background fluorescence from fish cells made interpretation difficult. The addition of 1/30 bovine

[3]Fluorescent anti-rabbit immunoglobulin (Sheep). Wellcome Reagents Ltd., Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, England.

[4]American Type Culture Collection catalogue of strains; No. 14715, 14417, and 19264, respectively.

[5]Bovine origin, Department of Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Sask.

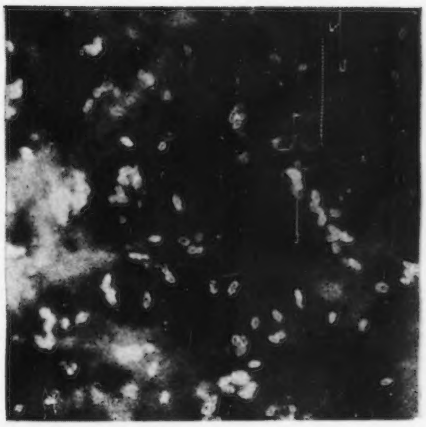

FIG. 1. RM bacteria in smear from kidney of experimentally infected rainbow trout. Indirect fluorescent antibody technique with rhodamine-labelled bovine albumin counterstain. ×750.

albumin rhodamine counter stain[6] rendered fish cells orange, allowing the specific green fluorescence to stand out brilliantly (Fig. 1). No specific fluorescence was observed on kidney smears from uninfected control fish.

Discussion — An indirect FAT for RM bacterium effectively detected the bacterium in washed cultures, broth cultures, and kidney impression smears from experimentally infected fish. The indirect method allows this technique to be easily adapted to any number of bacterial pathogens of fish using a single commercially prepared fluorescein-labelled antiglobulin. The reagents are stable for prolonged periods when stored frozen.

FAT can be advantageous over standard microbiologic methods when the sample is contaminated, the organisms nonviable, or when rapid diagnosis is desired (Cherry et al. 1960). Because of the high specificity and sensitivity of the technique, the pathogen can be detected without incubation to increase bacterial concentration.

Problems associated with FAT include loss of antigenicity due to handling procedures, masking of the antigen due to the location within the clinical specimen (i.e. mucous envelope or intracellular), enzymatic destruction, and nonspecific fluorescence (Cherry et al. 1960). In the present study, RM bacterium could be detected on fixed smears of washed and broth cultures stored at –23 C for more than 1 yr. Fresh smears kept in total darkness and handled in the absence of ultraviolet light can be stored at 20 C for up to 24 h without loss of fluorescence.

High concentrations of fluorescein have a self-quenching effect reducing the efficiency and clarity of the fluorescence (Cherry et al. 1960). Controlled dilution obtained the highest possible specific fluorescence and reduced complicating cross reactions with similar strains.

The addition of a rhodamine-labelled counterstain enhanced the efficacy of the technique by staining fish cells orange which contrasted with the green halo around the RM bacterium. The described FAT might be used as a rapid diagnostic test and might also be useful for the study of the pathogenesis of ERM. Studies are now under way to further evaluate the technique using experimentally infected fish.

[6]BBL Division of Becton, Dickinson and Company, Cockeysville, Md., 21030 USA.

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DDT and the Rugophilic Response of Settling Barnacles *Balanus improvisus*

N. MEITH-AVČIN[1]

Institute of Marine Sciences
University of North Carolina, Morehead City, N.C. 28557, USA

MEITH-AVČIN, N. 1974. DDT and the rugophilic response of settling barnacles *Balanus improvisus*. J. Fish. Res. Board Can. 31: 1960–1963.

Treatment of glass settling plates with DDT generally resulted in lowered settlement densities of the cyprids of *Balanus improvisus* and lowered indices of preference for roughened surfaces, indicating that DDT interferes with the cyprids' rugophilic response pattern to settlement surfaces.

MEITH-AVČIN, N. 1974. DDT and the rugophilic response of settling barnacles *Balanus improvisus*. J. Fish. Res. Board Can. 31: 1960–1963.

La colonisation des cypris de *Balanus improvisus* sur plaques de verre traitées au DDT est en général moins dense, avec préférence moins marquée pour les surfaces rendues rugueuses. Ceci indique que le DDT fait obstacle à la réaction rugophile des cypris aux surfaces de colonisation.

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SUBLETHAL amounts of DDT [1,1,1-trichloro 2,2-bis (*p*-chlorophenyl) ethane] have been shown to lower the tarsal chemical thresholds eliciting behavioral responses to sucrose solutions in DDT-sensitive houseflies (*Musca domestica*) and blowflies (*Phormia regina*) after their tarsi were kept in contact with a DDT-coated glass plate for 5 min (Smyth and Roys 1955). This effect was attributed to the increase in number of impulses delivered by each chemoreceptor at a given level of stimulation, later termed "repetitive afterdischarge" (Narahashi and Haas 1968), whereby a high-frequency chain of nervous impulses occurs in place of the normal electrophysiological pattern after treatment of the nerve with DDT. Yeager and Munson (1945) were the first to report that the sensory units of the nervous system in insects and crustaceans are most susceptible to these low-level DDT effects, and axonic, rather than synaptic, transmission is affected. Despite their finding that crustacean nerve is at least 10 times as sensitive as cockroach nerve, thus far there have been no efforts to determine possible behavioral effects of sublethal DDT on crustaceans in the field.

Crisp and Barnes (1954), using bakelite settling plates, demonstrated a consistent tactile response to surface contour by settling barnacle cyprids, which they termed a "rugophilic" response. They described the exploratory behavior of cyprids whereby holes, grooves, and scratches are the preferred settling sites. This rugotropism was shown to be independent of a light response.

In the present investigation, an examination was made of the effect of DDT, when applied to a glass surface, on the rugophilic response pattern of settling cyprids. It was the aim of the study to generalize the known DDT effects on sensory units of the arthropod nervous system to include tactile stimuli, and to test in the field the effects of low-level DDT application on the behavior of a common crustacean.

Materials and methods — Onto the surface of each of 50 15-cm square glass plates was sandblasted a checkerboard grid of 5.5-cm squares (Fig. 1). Plates were fastened onto a settling rack with galvanized steel wire threaded into a board of perforated masonite. This board was then placed into the rack with a solid plywood top over it to prevent light penetration. The rack was suspended in the water by a dock boat winch and cinderblocks were lowered into the rack for stability. The rack was maintained at a resting depth approximately 0.5 m below mean low tide and 1.0 m above the sediment surface. Glass plates were oriented facing the bottom to provide a suitable settling site

[1]Present address: Marine Biological Station, 66320 Portorož, Yugoslavia.

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for the subtidal barnacle *Balanus improvisus*, which settles from late winter to early summer in the Beaufort, N.C. area (McDougall 1943). The study was carried out from the boat dock of the Institute of Marine Sciences, on the northern shore of Bogue Sound.

In March of 1972, when settlement was low, plates were exposed in 3-day preliminary experiments to confirm cyprid preference for roughened surfaces in this species. Immediately after removal from the rack, a count of cyprids and metamorphosed individuals adhering to either type of surface was made and the preference ratios calculated (number on rough:number on smooth. When settlement rate increased in late April and May, DDT experiments were begun. Plates were dipped into known concentrations of DDT dissolved in acetone or ethanol (two solvents were used to expose synergistic effects). Half the control plates were dipped in solvent, the others left untreated. Six plates were exposed to settlement at one time: two experimental, two solvent controls, and two untreated controls. Exposure periods were 24 h.

In one experimental series in which DDT concentration was varied, plates were washed after exposure with 50 ml of petroleum ether and then analyzed for remaining DDT with a Varian Model 600-D gas chromatograph equipped with electron-capture detector. Before reuse, experimental plates were washed in acetone, 7X cleaning solution (Linbro Chemical Co., Inc., New Haven, Conn.), 10% HCl solution, and distilled water, and then autoclaved at 121 C (17 lb/in^2), to assure that no chemical residues from previously settled organisms remained to influence subsequent settlement.

During the 4-mo experimental period, two smaller plates were placed adjacent to the experimental plates to assure that settlement by barnacle species other than *B. improvisus* had been negligible during that period.

Results — Two preliminary 3-day plate exposures showed that *B. improvisus* preferred the rough, sandblasted surface of the plate (Table 1). Note that the ratio decreases with higher settlement densities, which is a consistent finding on control plates throughout the study. In the first experimental series of plate exposures, all plates were dipped in 1.0 g/liter DDT. Settled cyprids per plate square were counted and for each plate preference ratios calculated by pairing each rough square with a corresponding smooth square. Squares with like numbers in Fig. 1 were paired, yielding eight ratios per plate. Settlement was always highest toward the center of the plate, and this method of pairing compensated for this pattern. Settlers outside the central grid were not included in the count. In a plot of numbers calculated by this method, points representing DDT-treated plates are closer to the diagonal "line of no preference" for all settlement densities than are control points, although the two data classes converge at high settlement densities (Fig. 2A). A plot of data summed for each plate shows similar trends (Fig. 2B).

Regression lines (*a* and *b* in Fig. 2A and B) were calculated for control and experimental data. *t*-tests for comparison of slopes and *y*-intercepts were carried out, and differences determined to be significant ($P = 0.05$).

In the second series of plate experiments, in which plates were dipped into successively diluted DDT solutions (0.50, 0.25, 0.12, and 0.06 g/liter DDT), the amount of DDT remaining on the plates after the 24-h exposure period was determined and

TABLE 1. Number of barnacles settling on rough and smooth surfaces of each of eight plates from two 3-day exposure periods. Settlement preference ratios are calculated for total data from each period.

Exposure period	Plate no.	Rough	Smooth	Ratio R/S:1
1	1	44	0	
	2	23	3	
	3	15	0	
	4	86	1	
	Total	168	4	42:1
	1	274	22	
	2	357	32	
	3	156	2	
	4	143	3	
	Total	930	59	16:1

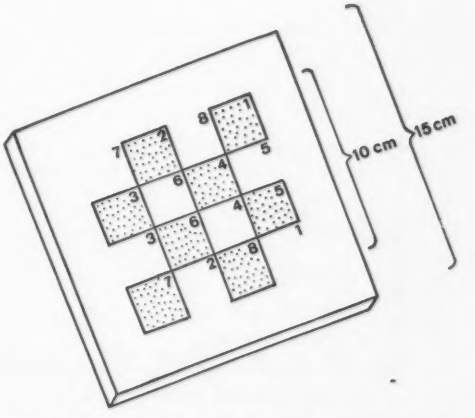

FIG. 1. Experimental settling plate, with checkerboard grid sandblasted onto the surface. Numbers refer to pairing order for calculation of preference ratios.

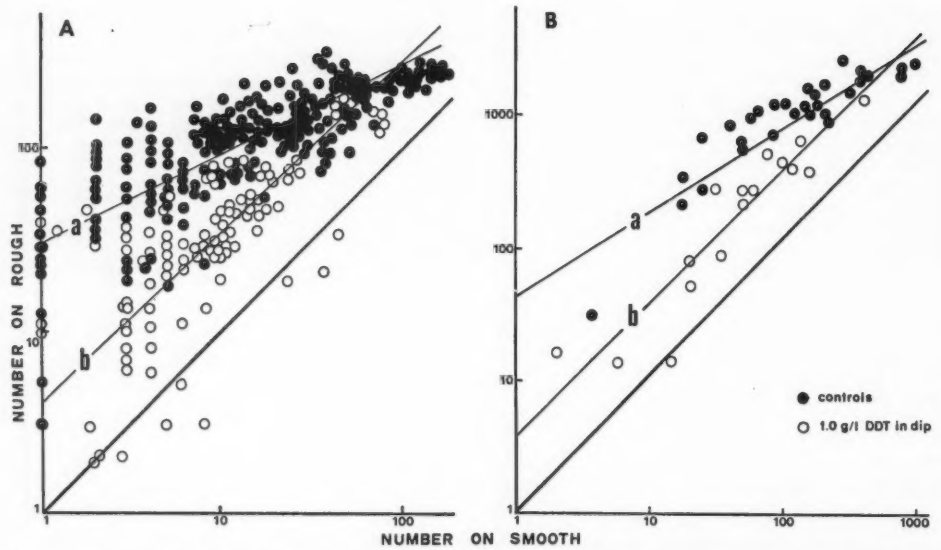

FIG. 2. A, Number of barnacles per rough square of each plate against number per corresponding smooth square. Data represents 48 plates, eight data points per plate; B, Number of barnacles on the sandblasted surface of one plate grid against number on the smooth surface. Each data point represents one plate, totaled from data represented in A.

plotted against the R/S:1 preference ratio for each plate. The data shows a clear trend toward a lowering of preference ratio with increasing DDT in plate wash. Note that below a level of approximately 0.03 μg/cm² DDT there are no ratios exceeding 10:1 (Fig. 3).

The second series of experiments was carried out during a period of occasionally stormy weather, and settlement densities varied drastically during this time, presumably due to strong currents washing the plates. Results from one calm exposure period during which settlement on control plates was satisfactorily high indicate the influence of DDT in reducing settlement density (Fig. 4). *t*-tests determined differences in means of successive data groups to be significant ($P = 0.05$).

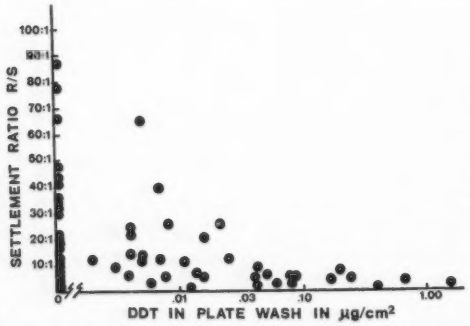

FIG. 3. Settlement ratio, R/S:1, for each series two plate against DDT remaining on plate at the end of the exposure period. Ratios were calculated from the total of rough and smooth settlers per plate, and control data are included on the ordinate.

Discussion — Figure 4 indicates that at certain concentrations DDT tends to inhibit settlement of cyprids. This is not an entirely consistent phenomenon, as Fig. 2A and B demonstrate, since some plates dipped in solutions of 1.0 g/liter DDT, the highest concentration used, had very high settlement densities. Although these were associated with control plates of even higher settlement, this is evidence that preference behavior is involved here rather than some mechanism which mechanically or physiologically prevents settlement on a DDT-coated surface at these treatment levels.

Table 1 supports the observation that cyprid preference for rough surfaces finds its greatest expression at relatively low settlement densities. At higher densities it is presumably the limitation of space for settlement that inhibits the behavioral mechanism involved in the expression of preference.

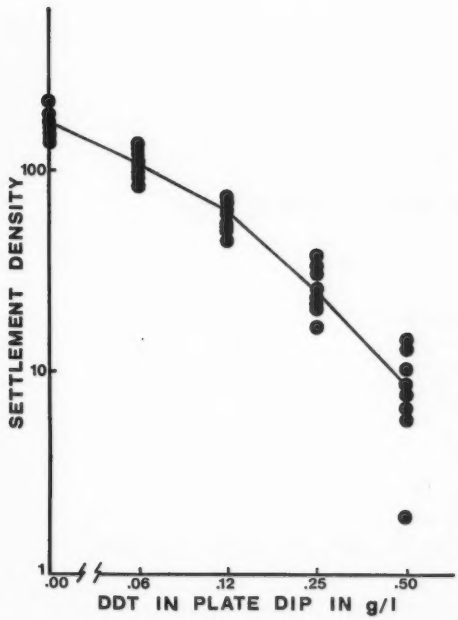

FIG. 4. Settlement density against DDT concentration of plate dip. Each point repesents settlers per pair of grid squares (one rough and one smooth) for six plates from one exposure period. Controls are shown on ordinate.

Since higher settlement densities on control plates tend to be associated with a decrease in the expression of preference for rough surfaces, the presence of a factor which would keep settlement at a relatively low level should lead, indirectly, to a higher preference ratio. Although DDT generally inhibits settlement (Fig. 4), it is also associated with a lowering of preference ratio (Fig. 2A, B and 3). This suggests that DDT influences cyprid behavior by interfering with the tactile discrimination known to be associated with the sensitive settling response.

Smyth and Roys (1955) attributed the response of their DDT-treated flies to a smooth surface to a possible effect on the mechanoreceptors involved in tactile discrimination, and from the present study a similar effect of DDT on the rugophilic response of barnacle cyprids is indicated. Whether the mechanism is one of physiological interference with the sensory processes involved in the rugophilic response pattern, or some other effect which inhibits the expression of preference, is not yet known. I have suggested a mechanism known from previous research, but the additional possibilities, such as mechanical interference by deposited DDT, indicate the necessity of further experimentation.

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Institutional Constraints in Fisheries Management

PETER C. THOMPSON

Department of the Environment, Fisheries and Marine Service, Freshwater Institute, Winnipeg, Man. R3T 2N6

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Abstract

THOMPSON, P. C. 1974. Institutional constraints in fisheries management. J. Fish. Res. Board Can. 31: 1965–1981.

The management of the Canadian fisheries is under the authority of both federal and provincial governments. While it is common to the Canadian confederation that both levels of government have dual roles in many areas of Canadian life, the situation with respect to the fisheries tends to be more complicated in the intricacies of federal–provincial jurisdiction. The result has led at times to a genuine confusion as to the management prerogatives over the Canadian fisheries.

In order to manage the resource it is considered requisite to have an understanding of the legislative prerogatives of the federal and provincial agencies responsible for Canadian fisheries. Such an understanding will serve to outline institutional constraints imposed upon fisheries managers.

This paper examines a unique aspect of the fisheries resource that being the nature of the governmental responsibilities in the management of the fisheries. This is achieved through the examination of the evolution of the Canadian federation, the constitution, the legal aspects of the fisheries, and the leading judicial decisions on Canadian fisheries.

Résumé

THOMPSON, P. C. 1974. Institutional constraints in fisheries management. J. Fish. Res. Board Can. 31: 1965–1981.

Les gouvernements fédéral et provinciaux ont tous deux juridiction sur les pêches au Canada. Bien que la Confédération canadienne offre plusieurs exemples d'une dualité de rôle des deux paliers de gouvernement dans bien des domaines de la vie, la situation dans les pêches a tendance à se compliquer par suite de la complexité des juridictions fédérales–provinciales. Il en est parfois résulté une véritable confusion quant aux prérogatives de gestion des pêches.

Il est essentiel à la gestion de la ressource de bien comprendre les prérogatives, définies par législation, des agences fédérales et provinciales responsables des pêches au Canada. Une telle compréhension va servir à mettre en relief les contraintes institutionelles imposées aux responsables de la gestion des pêches.

Cet article traite d'un aspect unique de la ressource, soit la nature des responsabilités gouvernementales vis-à-vis de la gestion des pêches. A cette fin, nous étudions l'évolution de la fédération canadienne, la constitution, les aspects légaux des pêches et les principales décisions des tribunaux en matière de pêches au Canada.

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Introduction

The vast inland area of Canada's "Central Region" is endowed with a large fisheries resource. Each year lakes in Ontario, Manitoba, Saskatchewan, Alberta, and the Northwest Territories produce sizable catches of freshwater fish which, in 1972, approached 39,000 metric tons with a landed value of some $15,000,000 (*10*, p. 33). Much of the catch has traditionally been exported. While the catch is relatively small beside other sources of Canadian fish protein, nevertheless, the commercial and recreational fisheries are an integral part of the economic and social life of the jurisdictions in which they are situated.

The inland fisheries are under the authority of both the federal and provincial governments. While it is a common part of the Canadian confederation that both the federal and provincial governments have dual roles in many areas of Canadian life, the situation with respect to the freshwater fisheries tends to be more complicated in the intricacies of federal–provincial jurisdiction. The result has led, at times, to a genuine confusion as to the management prerogatives over the fisheries of the inland waters. This situation has arisen as a result of the constitutional development of Canada and the adaption of British Common Law to the Canadian situation.

The purpose of this paper is to examine a unique aspect of the fisheries resource — the nature of the governmental prerogatives to manage and control the fisheries. To this end, it will be necessary to examine the evolution of the Canadian federation, the constitution as it is embodied in the British North America Act 1867, the legal aspects of the fisheries, and the leading judicial decisions that have contributed to the resolution of the legal–constitutional confusion in fisheries management.

Steps Leading to Confederation

"Whereas the Provinces of Canada, Nova Scotia and New Brunswick have expressed their desire to be federally united into one Dominion under the Crown of the United Kingdom of Great Britain and Ireland, with a constitution similar in principle to that of the United Kingdom..."(*18*)

The first paragraph of the preamble of the British North America Act 1867, expresses the wish that Canada's government be formed as a federal union, that is, that there be two orders of government. The concept of a federal system of government, at the time of confederation, involved a group of sovereign regional governments responsible for matters pertaining to the needs existing within their defined territories, and a central government responsible for matters of national concern, such as extra-territorial or interprovincial affairs. At the time of confederation, however, not all political thought was in favor of the provinces uniting in a federal union. There were many people who wanted Canada to unite legislatively, and create a government like that of the United Kingdom. The concept of legislative union has, as its focus, one government, which is solely responsible for all matters and needs of either a national or a local concern. The legislative type of government was quickly discarded as being impractical for Canada. The single government concept, it was felt, did not have the quality to respond to the diverse local needs that are generic to large countries. But the idea of the supremely sovereign central authority characterized in a legislative union held favor with many of the founders of the Canadian constitution. As a result, the federal system of government which evolved, incorporated some centralist characteristics of the legislative system of government.

Agreement on the general form of governmental system was followed by a debate regarding the division of responsibility, that could be struck between the two tiers of the federal system. Again, two schools of thought developed. One school, which had developed from the concept of the legislative union, favored a strong and powerful central government, and weaker subordinate provincial governments. The other school possessed a converse concept of federalism. These people desired a relatively weak central government designed from certain delegated powers, which were taken from strong and sovereign provincial governments, in order to give them the ability to speak as a single voice and handle problems of a common concern.

The British North America Act 1867, created a government combining these two styles of federalism. The Act fashioned a strong federal government with the power to legislate on important national topics including the power to disallow provincial legislation. In addition, the Act created strong provincial governments with explicitly stated powers to legislate and to collect revenues. Further, the provincial legislative prerogatives were constitutionally protected from encroachment by the central government ensuring that the provinces would carry on much as they had in the past as semiautonomous territories. As would be expected, any compromise with such diffuse intentions as these was bound to be somewhat ambiguous, and when put into actual practice was bound to lead to some confusion.

The federal union created by the BNA Act 1867, did not adequately delineate the boundaries of federal or provincial responsibility. This situation

has caused numerous conflicts between the two orders of government of which the administration and control of the inland fisheries became only one such area of dispute. Thus, the governments of the country were placed at the mercy of the courts of Canada and the United Kingdom to clarify the abstruse language that was used to define the extent of federal and provincial power, as well as to help determine some criteria to establish the constitution as a working document for the benefit of Canada.

The British North America Act 1867

The distribution of legislative powers between the central (federal) government and the provincial governments are specified in part VI of the Act. The legislative authority of the federal government is mainly delineated in section 91 of part VI of the BNA Act 1867 entitled the "Powers of Parliament" (*18*, p. 84):

"91. It shall be lawful for the Queen, by and with the Advice and Consent of the Senate and House of Commons, to make Laws for the Peace, Order and good Government of Canada, in relation to all Matters not coming within the classes of Subjects by this act assigned exclusively to the Legislatures of the Provinces; and for greater Certainty, but not so as to restrict the Generality of the foregoing Terms of this Section, it is hereby declared that (notwithstanding anything in this Act) the exclusive Legislative Authority of the Parliament of Canada extends to all Matters coming within the Classes of Subjects next hereinafter enumerated; that is to say..."

"Peace, order and government of all Matters" is a very broad and all encompassing statement. Within it could rest most matters of concern that were important to the uniting provinces in 1867. The expression is in a sense the residuary power of government, because there is little, if anything, concerning government that is not included in either peace for a nation, order within a nation, or good government of a nation. The framers of the BNA Act, recognized the wide extent to which this Parliamentary competence extended and included examples of subjects that were to illustrate and clarify, but not to restrict this-so-called residuary power. The enumerated subject classes of section 91 were by their nature, all the great and important topics of government. Each of the 29 subjects[1] listed, exemplify matters of common concern to two or more of uniting provinces (*17*, p. 18). These subject classes included among others, such topics as "postal service," "Militia, Military and Naval Service, and Defence"; "currency and coinage"; "navigation and shipping"; and "sea coast and inland fisheries." Thus a strong central government (Parliament) was created subject to the major constraint that provincial competence be respected. Section 91 concludes:

"And any Matter coming within any of the Classes of Subjects enumerated in this Section shall not be deemed to come within the Class of Matters of a local or private Nature comprised in the Enumeration of the Classes of Subjects by this Act assigned exclusively to the Legislatures of the Provinces."

This conclusion was intended, no doubt, to delineate the federal areas of competence, from the provincial areas that were to follow in section 92. In form it seems to say, that the federal concerns even though they may at first glance seem to resemble provincial concerns, do not and cannot be considered to fall within the provincial sphere of competence. This idea is evidenced by Mr Justice Gwynne of the Supreme Court of Canada, when passing his opinion on the subject of the meaning of this part of Section 91 which:

"in my opinion clearly is to exclude from the jurisdiction of the Local Legislatures the several subjects enumerated in the 92nd section, in so far as they related to or affect any of the matters enumerated in the 91st section." (*21*, p. 64)

The concluding paragraph of section 91 is probably one of the best examples of the ambiguity in the different parts of the Act. It should be noted that Gwynne's comments do not represent the only interpretations of the last paragraph of section 91. The Privy Council has ruled that when the federal government is legislating for peace, order and good government, the concluding words of section 91 should have no application unless the subject of the legislation is of national importance. In this situation the subject of legislation is the concern of the federal government because the matter is not of a local or private nature and therefore is beyond the jurisdiction of a province. Thus the residuary clause of section 91, should relate only to matters of utmost national concern (*4*, p. 360). These two points of view appear to be completely incongruous to each other. Yet at times each has been held to have some validity. It is the latter view today that would seem to be more in favor, as we shall see later.

Section 92 of the BNA Act 1867, specifies the exclusive powers of the provincial legislatures. The section is basically a list, but the subject areas are protected from federal government encroachment by section 91 which indicates that subjects were not

[1]Now 31 subject classes, with the addition head 2(a) in 1940 and head 1 in 1949.

to be the concern of the federal government. In contrast to the inventory of subjects in section 91, the provincial concerns were intended to be distinct and clear-cut, relating to matters of local concern. The subjects listed in section 92 included such topics as the management and sale of provincial lands; direct taxation in the province, such as the imposition of land taxes, which is in contrast to the unrestricted taxation powers of the federal government; and somewhat more nebulous but quite important terms such as civil rights and all matters of merely local or private nature.

Canada as a federal state needed not only a division of legislative powers but also a division of the prospective revenues of the four original uniting provinces. Thus part VIII sections 102–126 dictated how the "revenues; debts, assets, taxation" were to be handled under the new system of government. Two important sections, 109 and 117, concern us here. These sections vested in the four original provinces, Nova Scotia, New Brunswick, Quebec, and Ontario, the natural resources within their respective boundaries. The BNA Act 1867 also provided that British Columbia, Prince Edward Island, and Newfoundland should enter into Confederation and be given the same status as the four original provinces. Manitoba, Saskatchewan, and Alberta, did not have the same status as granted by sections 108 and 117 until the enactment of the BNA Act 1930. The revenues derived from the provincially owned lands were likely intended by the BNA Act 1867 to be primary source of provincial revenue. Thus the ownership of "all lands, mines, minerals and royalties" were vested in the provinces.

Constitutional Problems and Their Resolution

Parts VI and VIII of the Canadian Constitution are very important to the operation of federal union in that they pertain to the country's revenues and legislative responsibilities and to the distribution of these responsibilities between the governments of the federal state. The attempt at the allocation of legislative responsibilities does not seem to have been entirely successful. Primarily, the Act did not clearly define the extent of powers of parliament and the provincial legislatures. Secondly the Act failed to completely consider conflicts arrising between the levels of government within the federal state or to consider amelioration of conflicts of, firstly, a federal–provincial nature, and secondly of an interprovincial nature (*24*). Thus it fell upon the courts to clarify the language of the law, and to settle constitutional disputes. The Supreme Court of Canada today is the final court of appeal and is therefore the official interpreter of the BNA Act 1867. However, this has not always been so. Until 1949 the Judicial Committee of the Privy Council of the United Kingdom ranked as the highest court in the land. This judicial board was set up in England as the court of ultimate appeal for the British Empire. As a result the Privy Council was largely responsible for many of the interpretations of the BNA Act 1867, and for the resolution of the disputes arising from different interpretations of the Act.

Most of us are familiar with the phrase "provincial rights." The struggle by the provinces for their rights and privileges is not new. The struggle started with confederation in 1867 and has continued since then. The conflict between the federal government and provinces has occurred because of the ambiguity in our constitution. The courts have attempted to clear up the problem areas and while it is recognized that no cardinal rule can be developed to resolve all federal–provincial disputes over areas of common concern, there do exist certain canon principles that serve to help mitigate major conflicts and keep the two orders of government to their respective areas of concern.

The resolution of federal–provincial disputes arising from different interpretations of the BNA Act 1867 were heavily influenced by the outcome of the confrontation over the provincial rights. Judicial decisions would be quite different on the one hand, where the provinces were considered to be subordinate governments of the federal governments, to a decision made on the premise that the two levels of government were more or less equal partners. The courts, in deciding the extent of provincial rights, were forced to articulate the basic attributes of the federal and provincial governments. Some of the criteria used in defining the stature of the provinces were stated by Mr Justice T. J. J. Loranger in 1884:

"At the time of Confederation, all legislative and executive power, legal attributes, public property and revenues, that are now the appanage of the central government and the provinces, belonged to the latter... In constituting themselves into a confederation, the provinces did not intend to renounce, and in fact never did renounce their autonomy. This autonomy with their rights, powers and prerogatives they expressly preserved for all that concerns their internal government; by forming themselves into a federal association, under political and legislative aspects, they formed a central government, only for interprovincial objects, and, far from these provincial powers that has arisen the federal government, to which the provinces have ceded a portion of their rights, property and revenues." (*16*, p. 7–8)

This statement illustrates the basic principle that the provinces were not the subordinate creatures in

confederation but that they were on a more or less equal footing with the federal government. The concept that the provinces do have "rights" is of an important nature. The federal government cannot expect to win constitutional disputes primarily because it is the superior government. The Privy Council recognized the partnership of governments in the Canadian confederation and often expressed the view of equality among the federal and provincial governments. For example:

"The object of the Act was neither to weld the provinces into one, nor to subordinate provincial governments to a central authority, but to create a federal government in which they should all be represented, entrusted with the exclusive administration of affairs in which they had a common interest, each province retaining its independence and autonomy. That object was accomplished by distributing, between the Dominion and the provinces, all powers executive and legislative, and all public property and revenues which had previously belonged to the provinces; so that the Dominion Government should be vested with such of these powers, property, and revenues as were necessary for the due performance of its constitutional functions, and that the remainder should be retained by the provinces for the purposes of provincial government. But in so far as regards those matters which, by sect. 92, are specially reserved for provincial legislation, the legislation of each province continues to be free from the control of the Dominion, and as supreme as it was before the passing of the Act." (*15*, p. 441)

Many of the early constitutional battles between the federal and provincial governments centered around the theme of provincial "crown" prerogatives. The concept of crown prerogatives is a major factor in the assertion of provincial rights in Canada. The prerogatives of course have evolved from the "divine rights of monarchs" and under the British system involve a large number of discretionary powers held by government. These powers include the right to claim as the property of the crown (in trust for the people) escheats and forfeitures (these involve property and assets reverting to the crown and imply that all property rights stem from grants by the crown) the precious metals, gold and silver of all properties in the nations, and the unclaimed or ungranted land of the nations. In the United Kingdom the crown prerogatives are vested in one government rather than the two orders of government in our federal system.

After confederation in 1867 the central government held that the administration of the crown prerogatives was vested solely in the federal government through the Governor-General in Council. Further the federal government held that the BNA Act 1867 did not bestow the crown prerogatives on the provincial governments through their respective Lieutenant-Governors. The contention was that the BNA Act 1867 severed any direct connection between the crown and the Lieutenant-Governor (*8*, p. 107) and that the powers of the Lieutenant-Governors, and the provincial cabinets were delegated powers from the Governor-General. This view of the Lieutenant-Governors being mere agents of the federal government was advocated in support of the federal government by the Colonial Office in London (*17*, p. 91). The result of a view such as this, would of course mean rightful claim by the federal government to escheats and publicly held lands not directly granted to the provinces in the BNA Act 1867 and a resulting subordination of the provincial governments. The courts of course, in several decisions, upheld the claim for provincial rights on the grounds that the provinces were not subordinate governments as the lack of crown prerogatives would have, but that the provinces were in a manner of speaking, independent and autonomous.

As has been mentioned, Part VI of the BNA Act 1867 listed a number of subjects defining the spheres of influence that the two orders of government were to be responsible for. There are areas of legislative jurisdiction where it is clear that the matter concerns the federal government and the provinces have no business in the matter at all. National Defense and Military is one of these areas. There are also matters of provincial competence that the federal government has no authority to act upon, except through its unlimited taxing power and the rights to spend money as it sees fit, which do not suggest any authority over an area of provincial competence. These subject classes include the sale of provincial timber and wood, and education in the provinces. However, there are other subjects of legislative jurisdiction where the authority over the matter is not clear. As a result confusion and dispute have developed between the two levels of government. In many cases the courts have been left to clarify these areas of dispute. One of the primary relationships pointed out by the courts is that a concurrent authority can and must exist between federal–provincial legislative jurisdiction. It has been stated more than once that:

"...the two sections 91 and 92 are not mutually exclusive and that the provisions may overlap... (*9*)

Thus we see that both legislatures can act on a single subject at the same time. The act does not imply that if one level of government seems to have authority that the other level of government must necessarily be excluded from taking action on the subject matter. The federal government has on occasion attempted to claim that BNA makes no room for concurrent jurisdiction in section 91 and

92 because of the use of the word 'exclusive' in referring to the governments' respective areas of legislative jurisdiction. There are, however, areas where the overlapping of federal–provincial legislative jurisdiction is restricted. The courts have gone to great lengths to spell out the extent to which federal–provincial legislation may be concurrent and where and when one order of government's legislation will supercede the other government's legislation. Thus there are areas where there is seemingly overlapping jurisdiction but in effect there can be none.

"... But section 91 expressly declares that notwithstanding anything in this act, the exclusive legislative authority of the Parliament of Canada shall extend to all matters, coming within the enumerated classes; which plainly indicates that the legislation of Parliament, so long as it strictly relates to those matters is to be of paramount authority..." (*23*, p. 45)

It would appear, then, that the federal parliament may enact any piece of legislation even if the enactment encroaches upon a field of provincial legislative jurisdiction so long as the intent of the statute pertains only to the field of federal competence and the intent is not to usurp provincial powers enumerated in section 92. This rule seems to affect only the federal legislative jurisdiction described by the 29 subject classes of the BNA Act 1867. Thus when the Parliament of Canada enacts a statute which is clearly within federal competence outlined by section 91 it will be of paramount authority. Provincial legislation on a subject that strictly relates to a subject class of section 91 will have no validity.

Federal statutes may also stretch beyond a strict relationship to the enumerated subjects into areas which may clearly be within provincial competence. This can happen, however, only when such legislation is required to ensure that federal legislation on an enumerated subject will be observed. Such encroachment, then, into a topic of provincial competence must be necessarily incidental to an area of federal competence. This does not mean that provincial legislation on that topic will be invalid, only that it will have no force if it conflicts with the federal enactment.

The federal government has also been given by the BNA Act 1867 the residuary legislative authority to enact on matters of peace, order, and good government of Canada. This power as stated was likely intended to be the authority by which Canada would be effectively governed. However, the courts, in ruling that the provinces did have their provincial rights and that the provinces were not to be subordinate to the central government, perceived that the unrestricted use of peace, order, and good government by the federal government could adversely effect the independence and autonomy of the provinces. As a result the courts could have ruled that federal legislation under peace, order, and good government must attain a stature of national importance to be valid. This point was stated in 1896 by Lord Watson:

"There may, therefore, be matters not included in the enumeration, upon which the Parliament of Canada has power to legislate, because they concern the peace, order, and good government of the Dominion. But to those matters which are not specified among the enumerated subjects of legislation, the exception from section 92, which is enacted by the concluding words of section 91, has no application; and, in legislating with regard to such matters, the Dominion Parliament has no authority to encroach upon any class of subjects which is exclusively assigned to provincial legislatures by section 92. These enactments appear to their Lordships to indicate that the exercise of legislative power by the Parliament of Canada, in regard to all matters not enumerated in section 91, ought to be strictly confined to such matters as are unquestionably of Canada interest and importance, and ought not to trench upon provincial legislation with respect to any of the classes of subjects enumerated in section 92." (*4*, p. 360)

The solution of possible dispute arising out of seemingly overlapping federal–provincial jurisdiction is again provided for. The federal government is forbidden from trenching on provincial matters listed by section 92 with its legislative power from peace, order, and good government. Thus federal legislation not arising from the enumerated subject classes, will have no validity unless it attains great national importance, when it relates to provincial topics. Here again, when federal legislation overlaps into a field of provincial competence, that is, that the legislation is of national importance, the provincial legislation will be allowed to exist as long as it is not repugnant to the intent of the allowable federal legislation. If repugnancy exists then federal legislation will prevail.

Thus when the BNA Act 1867 failed to delineate the extent of the powers of parliament and the provincial legislatures, the courts of Canada have interpreted the Act and handed in their judgements. The many decisions down through the years, attempting to resolve constitutional disputes, have stated the above principles to follow in settling general areas of confusion in the Canadian constitution. These canons were summarized by Lord Tomlin in the B.C. Fish Canneries reference in 1929 and serve as a good summation of this decision.

"(1) The legislation of the Parliament of the Dominion, so long as it strictly relates to subjects of legislation expressly enumerated in s. 91, is of

paramount authority even though it trenches upon matters assigned to the provincial legislature by s. 92. (2) The general power of legislation conferred upon the Parliament of the Dominion by s. 91 of the Act in supplement of the power to legislate upon the subjects expressly enumerated must be strictly confined to such matters as are unquestionably of national interest and importance, and must not trench on any of the subjects enumerated in s. 92 as within the scope of provincial legislation unless these matters have attained such dimensions as to affect the body politic of the Dominion. (3) It is within the competence of the Dominion Parliament to provide for matters which, though otherwise within the legislative competence of the provincial legislature, are necessarily incidental to effective legislation by the Parliament of the Dominion upon a subject of legislation expressly enumerated in s. 91. (4) There can be a domain in which provincial and Dominion legislation may overlap in which case neither legislation will be ultra vires if the field is clear, but if the field is not clear and the two legislations meet the Dominion legislation must prevail." (*2*, p. 196)

Fisheries

Thus the Canadian constitution in the BNA Act 1867 and its interpretation in the courts has distributed the legislative authority between the two orders of government of our federal state. There are some subjects where one of the governments is clearly responsible for the sole enactment of statutes, and there are other matters of concern where both orders of government may have concurrent authority. The water resources of Canada, the coastal fisheries, and the inland fisheries are examples where there is a dual role played by the two levels of government in the management and regulation of the resource. As would be expected the BNA Act 1867 did not resolve the distinct roles that each order of government had over either the fishery or the total water resource. This lack of distinction between federal–provincial roles in fisheries jurisdiction has precipitated a number of constitutional disputes requiring the courts of Canada to resolve the confusion over fisheries authority, not to mention other disputes over the other aspects of the water resource. With the passing of years the federal–provincial roles in fisheries management have become more distinct. There are, however, several important principles which merit discussion if the legal–constitutional aspects of the central region fishery is to be better understood.

The BNA Act 1867 places the seacoast and inland fishery as one of the enumerated subjects to section 91, under the *exclusive legislative authority* of the federal government. Accordingly, with the responsibility extending to all matters pertaining to seacoast and inland fishery, it would seem that only the federal government should have the exclusive control over the resource. The question then arises, why do the provinces have management responsibilities in fisheries when the constitution specifically states that the seacoast and inland fisheries are within exclusive federal legislative competence? Part of the answer lies in understanding what the term fishery means at common law and how this relates to the BNA Act 1867.

A fishery is more than just a place where fish are present. The Fisheries Act (R.S.C. 1970, chapter F-14) makes the distinction that a fishery is an "area, locality, place, or station" where fishing equipment is used or may be used to capture fish. At law, however, there is another very important aspect to a fishery. This is, that it is a right or liberty of taking fish (*6*). The general character of all fisheries is that the fish are the produce of the land, over which the water, in which the fish exist, flows (*11*, p. 167). This does not mean that the fish are property, as they can only become property upon capture and placed in a person's possession. Moreover, the fishery or more properly the right to capture fish may belong to the owner of the bed of the watercourse. This implies that there is attached to a fishery at least some of the aspects of ownership. It is possible to define two basic groups of common law fishery, the proprietary fishery and the public right of fishery, each of which represents different types of tenancy.

The proprietary fishery is characterized by an exclusive right to fish. The right to fish or the ownership of the fishery is directly connected to the ownership of the bed of the watercourse. Thus in the west, the Prairie pothole lakes are often proprietary fisheries, in the sense that the owner of the farm who, as the owner of the bed of the pond, can lawfully exclude people from fishing in his lake. The crown in the right of the provinces is also an owner of proprietary fisheries. The provincial governments, in most cases, did not grant the beds of rivers and lakes to its grantees. Thus in Lake Winnipeg, there is an exclusive right to fish which is held by the crown in the right of the Province of Manitoba. This points out another facet of proprietary fisheries. The fishery or right of fishing, may be severed from the ownership of the soil and given by grant or prescription to a second party. The fishery is then like a franchise which is an exclusive right to fish with no rights to the bed of the waters. The owner of the proprietary fishing may grant the privilege to fish on his property to whom he sees fit, by lease or license. In Canada proprietary fisheries exist only in the nontidal, inland waters.

The public right of fishery as in the case of the proprietary fishery treats the fish as the produce of the land. However, the relationship between the ownership of the bed of the watercourse and the ownership of the fishery (fishery rights) has been annulled. The exclusive right of fishing which is unique to the proprietary fishery is replaced with the right of the public to participate in the fishery. The fish remains the profits of the land, not becoming property until capture as before the proprietary nature of fisheries is removed. The public right of fishery means that there is a general and common right of citizens to participate in the capture of fish and that the right to fish cannot be limited to the exclusive privilege of a person or a group of persons. This has not always been the case. Prior to the signing of the Magna Carta, the King could grant the exclusive right to fish to anyone he wished (*1*, p. 170). The Magna Carta, however, restricted the crown from exercising this crown prerogative and established the public's right to fish. Parliament may and has granted fishing rights through the grant of leases or licenses as a regulatory control over the public right of fishing but this does not approach the kind of property rights in proprietary fishery, i.e. the right to fish if granted would not be inheritable or saleable. The public right to fish in Canada can exist only in tidal waters. The public right of fishery was established in England in navigable waters. At common law navigable waters, where a public right exists, are only those in which the tide ebbs and flows. Thus the public right of fishery exists where there is no exclusive or absolute right for anyone to fish that is on both the east and west coasts, Hudson's Bay and James Bay, and in the Arctic Ocean.

The provinces of Canada by virtue of sections 109 and 117 of the BNA Act 1867 were vested with the ownership of much of the land of the country. Further, by section 92 they were given the authority to legislate with respect to property, and to the management and sale of the provincial lands. The nature of proprietary fisheries, that is, their common law attachment to the bed of the watercourse, clearly puts these fisheries in the sphere of provincial competence. It appears that the framers of the BNA Act 1867 failed to recognize that by granting only the exclusive legislative jurisdiction of the seacoast and inland fisheries to the responsibility of the federal government, and not including with that grant the ownership of the beds of the nontidal watercourses in Canada, they were creating two jurisdictions responsible for the same resource. This fact is indicated by the assumption by the federal government in the years immediately following confederation of the complete control over all the fisheries in Canada (*19*, p. 799). Under the authority of the Fisheries Act of 1868 the Department of Marine and Fisheries granted fisheries leases and licenses in both tidal and nontidal waters not recognizing that because the nontidal fisheries were in most cases property of the province, the Minister of Fisheries in granting leases and licenses was overstepping the legal authority that the BNA Act had placed in the federal government. Thus the BNA Act 1867 created with respect to the inland (nontidal) fisheries a resource that, in the eyes of the law, had two more or less distinct aspects to it, the legislative jurisdiction and the proprietary rights (*3*, p. 709). The result of the situation is that the two levels of government both exercise control over fisheries in Canada. The federal government exercises legislative jurisdiction over both the seacoast and inland fisheries but cannot exercise any proprietary rights over fisheries that it does not own. Today in the N.W.T. and in the National Parks the federal government has complete jurisdiction over every aspect of them because they are the owners of that resource. The provinces may exercise proprietary rights over fisheries that are vested in them. It must be stressed that the proprietary rights can exist only in the inland fisheries because the property aspects of fisheries in tidal waters are no longer applicable, having been replaced by the public right to fish. Because of the failure to recognize the legal implications attached to fisheries and the resulting failure to place the complete control of fisheries under one jurisdiction, as was the probable intention of the BNA Act 1867, and the difficulties in defining the limits of federal–provincial authority, the fisheries of the seacoasts and the inland waters went through a prolonged period of dispute and contention as to which level of government would have control over the management of the resource.

The confusion over the federal–provincial management prerogatives of Canada's fisheries resources has been clarified through the court's arbitration and interpretation of the Canadian constitution. The judicial cases and references that follow are intended to illustrate and explain the management responsibilities of the federal and provincial governments as they exist today.

The Queen v Robertson 1882

This was the first case involving fisheries to go to the Supreme Court of Canada. In 1874 the Minister granted to a Mr Robertson under the authority of the Fisheries Act of 1868 a nine-year lease on a section of the Miramichi River in New Brunswick for the purpose of salmon angling. Subsequently, his ownership of the fishery lease was contested by persons claiming the exclusive ownership of the fishing of that portion of the river, including Mr

Robertson's leasehold. The courts of New Brunswick did not recognize his lease and Mr Robertson petitioned the federal government for compensation for the loss of his fishing privileges. The appeal, by the federal government, against the verdict granted by the Exchequer Court of Canada, in Mr Robertson's favor, was heard in the Supreme Court of Canada.

The Queen v *Robertson* case raised the initial questions that were to challenge the authority of the federal government to have the complete administration and control of all the fisheries of Canada. The primary point raised by the Supreme Court's decision was that in Canada there did exist different rights of fishing in the tidal portions of rivers than existed in the nontidal portions of rivers.

The court determined that in Canada the public right of fishing did not exist in nontidal portions of rivers. They held that an exclusive right to fish in nontidal streams existed with the riparian owners, i.e. the owner of land bounded generally upon a stream of water. The proprietary fishery was held to be established because, unless specifically excepted from the grant of land, the bed of the nontidal stream was vested in the proprietorship of the riparian grantee. As such the Supreme Court contended that it was beyond the authority of the federal government to grant leases or licenses to fish in nontidal portions of rivers, as the exclusive right to fish existed in riparian proprietors. In the case of the tidal parts of rivers it was judged to be within the authority of the Minister of Fisheries to regulate the public right of fishing by means including the grant of leases and licenses.

The federal government also asked the Supreme Court whether or not it was entitled to grant fisheries leases and licenses where nontidal streams passed through ungranted lands. Chief Justice Ritchie indicated the answer in the following manner.

"I reiterate what I on a former occasion intimated, that at the time of the union the entire control, management and disposition of the crown lands, and the proceeds of the public domain, were confided to the executive administration of the provincial governments as representing the crown for the benefit of the provinces respectively, and to the legislative actions of the provincial legislatures, so that the crown lands, though standing in the name of the Queen, were, with their accessories and incidents, to all intents and purposes the public property of the respective provinces in which they were situate; and this property, the Imperial Act, by clear unambiguous language, has, as we have seen, declared shall after confederation continue to be the property of the provinces; and I cannot discover any intention to take from provincial legislatures all legislative power over property and civil rights to deprive the province or individuals of their right of property therein, and to transfer the same or the enjoyment thereof to others as the license in question affects to do." (*21*, p. 123)

The point made is that the ungranted (crown) lands lying within a province were vested not in the federal government by the BNA Act 1867 but by the crown for the benefit of the province, in this case the province of New Brunswick. Thus in New Brunwick there did exist an exclusive right to fish on nontidal waters flowing through ungranted lands. Further, the exclusive right to fish belonged to the crown, as the owner of the ungranted lands, in trusteeship for the Province of New Brunswick.

The Chief Justice raised some other interesting points that were not to be settled until a later date. Firstly, that the entire control and management of crown lands, and by implication fisheries, was before confederation in the hands of the provinces and that the BNA Act did not convey this right to the federal government. Secondly, the point was raised that the provinces were entitled to have some legislative authority in the property and civil rights of their proprietary fisheries. However, the court did emphasize that the federal government has the general power of regulating and protecting all fisheries under the terms of the BNA Act 1867. As was aptly put by Mr Justice Fournier at the time, it was to the general interest of the public that there be a general fishing law, which would ensure that the fisheries were not exploited to the point of ruin, but that the fisheries be policed in such a manner that they were to be developed and conserved for the general profit of Canada (*21*, p. 140).

After this decision by the Supreme Court the provinces of Quebec and New Brunswick acquired the function of leasing salmon angling rights in the nontidal reaches of rivers within their jurisdictions. It would appear likely that because the case pertained specifically to the leasing of salmon-angling privileges, the federal government did not entertain any thought of releasing any further control over the fisheries of inland lakes or rivers to the provinces. Rather, the federal government continued to exercise complete control over fisheries. It would also appear that the other provinces did not see any immediate advantage in exercising their proprietary rights over their fisheries as did New Brunswick and Quebec (*20*, p. 2). As a result it was not until 16 years later that the general situation with respect to provincial proprietary rights and federal legislative jurisdiction was further clarified.

The Fisheries Reference 1898

The decision in *The Queen* v *Robertson* of 1882 by the Supreme Court of Canada initiated the

disgruntlement among the provinces over the federal government's jurisdictional control of the inland fisheries. Ontario was in the period of the 1880s the leading province in the contest for provincial rights (*17*, p. 54). The federal government's control of fisheries, especially over the Great Lakes Fisheries and the sizable revenues collected from the fisheries, was probably a large source of vexation to the province. Canada Department of Marine and Fisheries, *Annual Report* 1893, reported that Ontario revenues from licenses, rents, and fines for the year 1884–85 totalled $11,914.37 out of a total Canadian revenue from the same sources of $44,911.06. More than likely as an attempt to capture revenues from Ontario fisheries the Ontario legislature enacted in 1885 the Ontario Fisheries Act (48 Victoria, chapter 9). The Act reappeared in the Revised Statutes in 1887 as "An Act to Regulate the Fisheries of this Province." (R.S.O. 1887, chapter 32). In that year the Ontario Department of Crown Lands reported revenues from licensing as being very meager (*11*). It would appear that provincial regulations and licensing schemes were neglected by the provincial government and ignored by the fishermen. One of the Ontario fisheries regulations of 1888 indicates this, in reporting that parties holding Ontario leases and licenses could have no recourse to the Ontario government for loss or hindrance of their privileges by an act of the Government of Canada (*11*). It does seem, however, that the government was attempting to dispute federal government control over fisheries.

The matter came to boil in 1892 when the Ontario government enacted stiffer legislation attempting to regulate the manner of fishing in the province, as well as attempting to license and collect fees (An Act for the Protection of Provincial Fisheries, 55 Victoria, chapter 10). This action, no doubt, was initiated by a desire to collect most of the revenues from fishing in the province but also because of the findings of the Ontario Game and Fish Commission of 1890–91. The report of the Commission mentioned that their study of Ontario fisheries was the most difficult they undertook because of the constitutional nature of fisheries and that they found it difficult to make recommendations as to what to do about them (*12*). This period was at the beginning of the conservation movement that was gaining momentum in North America, and the commission expressed alarm at the depletion of fish in some areas and possible justifiable consternation at the lack of federal response to the problems of depletion of fish stocks. It appears from the report of the Ontario Game and Fish Commission that at this time fisherman were experiencing decreasing catches and that the federal government responded by increasing the length of certain open seasons. Thus it appears that the Ontario government's action was not only motivated by a desire to assert its rights but also motivated by the desire to conserve the province's fisheries.

Ontario's outright challenge to the federal government's assumed responsibility over the inland fisheries initiated the action to have a test case put before the courts to settle the matter. The case went before the Supreme Court of Canada in 1896 and the appeal by the Attorney-General of Canada went before the Judicial Committee of the Privy Council in 1898. The decision of the Privy Council changed the nature of government involvement in the inland fisheries from one where the federal government had explicit control, to a situation where there was a shared federal–provincial responsibility over the inland fisheries. Thus it was determined that the BNA Act 1867 had conferred to the federal government only legislative jurisdiction over fisheries:

"Their Lordships are of opinion that the 91st section of the British North America Act did not convey to the Dominion of Canada any proprietary rights in relation to fisheries. Their Lordships have already noticed the distinction which must be borne in mind between rights of property and legislative jurisdiction. It was the latter only which was conferred under the heading, 'Sea-Coast and Inland Fisheries' in s. 91." (*3*, p. 712)

Thus the federal government by licensing and leasing fisheries in the nontidal waters was not legislating within its sphere of jurisdiction, but was in substance usurping provincial authority by legislating on property and civil rights in fishing.

I indicated earlier that nontidal fisheries are proprietary fisheries and are clearly within the sphere of provincial competence. This becomes more evident when it is recognized that matters of property of the inland fisheries are outside of federal jurisdiction. The Privy Council in its 1898 decision attempted to define provincial jurisdiction over the property in fisheries:

"For example, provisions prescribing the mode in which a private fishery is to be conveyed or otherwise disposed of, and the rights of succession in respect of it, would be properly treated as falling under the heading 'Property and Civil Rights' within s. 92, and not as in the class 'Fisheries' within the meaning of s. 91. So, too, the terms and conditions upon which the fisheries which are the property of the province may be granted, leased, or otherwise disposed of, and the right which consistently with any general regulations respecting fisheries enacted by the Dominion Parliament may be conferred therein, appear proper subjects for provincial legislation, either under class 5 of s. 92. 'The Management and Sale of Public Lands' or under the class 'Prop-

erty and Civil Rights.' Such legislation deals directly with property its disposal, and the rights to be enjoyed in respect of it..." (*3*, p. 716)

The province's proprietary rights in fishing allow the provinces to legislate with respect to the conveyance, disposal, and rights of succession of several fisheries within the province. Further, the power to legislate carries with it, for the province, the power to administer and control the resource as would be expected to be exercised by a private owner (*13*, p. 167). This may involve the granting, leasing, and licensing of provincial fisheries subject to any terms or conditions as the province may choose to impose. Of course as intimated by the Privy Council, acts of this type must be consistent with federal legislation. Repugnancy between federal and provincial legislation touching on the same matters has the effect of making provincial legislation invalid.

The Privy Council ruled on the validity of the Province of Ontario's 'An Act for the Protection of Provincial Fisheries' which attempted to regulate the manner of fishing. The board's judgement contended that the provisions of the Ontario Act in question were clearly within the federal government's exclusive competence and therefore the provinces were not entitled to regulate the manner of fishing as Ontario had attempted. The Judicial Committee held that the federal government's legislative jurisdiction encompassed the setting of open and closed seasons for fishing, the equipment which can be used to capture fish, and in general the right to pass legislation necessary for the protection and preservation of fish and fisheries within Canada.

Therefore it appears that the federal government may legislate for the regulation, protection, and preservation of the sea coast and inland fisheries but they may not grant any right to fish in waters where the exclusive right of fishing exists in the crown in right of the province. Nor it seems, may the federal government legislate with respect to the property in the inland fisheries (the proprietary fisheries) as that would constitute trenching on the exclusive provincial competence over property. However, the provinces, in fisheries, may not legislate for the regulation, protection, and preservation of fisheries as that would constitute an entrenchment on a field of exclusive federal jurisdiction. The provinces must be content to involve themselves with the disposal of fisheries, prescribing conditions that grants of fisheries should be subject to, and the administration of the resource.

The Privy Council recognized that the federal legislative jurisdiction was powerful in that short of exercising property rights with federal legislation, the federal government could restrict the provincial government's legislative competence when such was in the same sphere as topics necessarily incidental to the federal jurisdiction over the seacoast and inland fisheries.

"At the same time it must be remembered that the power to legislate in relation to fisheries does necessarily to a certain extent enable the legislature so empowered to affect proprietary rights. An enactment, for example prescribing the times of year during which fishing is to be allowed or the instruments which may be empowered for the purpose (which it was admitted the Dominion Legislature was empowered to pass), might very seriously touch the exercise of proprietary rights, and the extent, character, and scope of such legislation is left entirely to the Dominion Legislature. The suggestion that the power might be abused so as to amount to a partical confiscation of property does not warrant the imposition by the Courts of any limit upon the absolute power of legislation conferred. The supreme legislative power in relation to any subject-matter is always capable of abuse, but is not to be assumed that it will be improperly used; if it is the only remedy is an appeal to those by whom the legislature is elected." (*3*, p. 713)

Thus the legislative powers of the federal government are such that, for example, a fishery could be closed down completely, depriving a proprietor of his rights to capture fish. The federal legislative jurisdiction over fisheries can touch upon many subjects including property and civil rights. However, it appears that such legislation would have to be shown to be essential to the discharge federal responsibility over fisheries. The Courts, in a case such as this would want to leave no doubt whatsoever that an action as above was necessarily incidental for the conservation of the inland fisherman.

British Columbia Fishing Rights 1914

Following the Privy Council's judgement in the fisheries Case 1898, British Columbia and the Maritime Provinces began to assert that in view of decision handed down, they should be given control of the administration of the tidal fisheries to the three-mile limit. The Provinces contended that there was property interest in fisheries to the three-mile limit of the Canadian coastal waters and the administrative jurisdiction of such belonged to the provinces (*20*, p. 9). The federal government, of course, held that while the provinces had proprietary rights in the inland fisheries, when it came to the seacoast fisheries there existed no interest of property in fisheries and as such the seacoast fisheries were entirely within the control of the federal govern-

ment. This dispute was settled by a reference before the Judicial Committee of the Privy Council in 1914.

The Privy Council's decision considered fisheries rights as a species of property as they related to the federal and provincial legislative powers. Briefly the decision contended that in tidal waters the public had been given the right to fish and that the relation between the ownership of the fisheries and the ownership of the solum, no longer had any bearing. As such it was agreed that since there was no proprietary nature in tidal fisheries, the provinces had no jurisdiction whatsoever over the fisheries of the seacoasts or the tidal estuaries. The federal government, however, due to its constitutional responsibility for the seacoast and inland fisheries, has the legislative power to regulate the exercise of the pubic right of fishing where those rights exist.

The situation in the nontidal inland waters as intimated in the Privy Council decision of 1898 is different. In nontidal waters fishing is a subject of property and as such has an owner. Since no public right to fish has been established in the inland waters the federal government must concede to the property rights in these fisheries where the federal government does not own the solum itself. In this case then as the provinces are the owners of most of the inland fisheries, there is control exercised by the two orders of government.

This reference clarified and reiterated the principles that had been set out in 1898. The result was that it set out clearly the role of the federal government in its complete jurisdiction over the fisheries of the seacoasts and in doing so the judgement of the Privy Council, again attempted to make clear distinction between the federal–provincial responsibilities in the inland fisheries of Canada.

The British Columbia Fish Canneries Reference 1929

This case before the Supreme Court of Canada and the Privy Council involved the constitutional validity of a section of the Fisheries Act of 1914. The Act attempted to introduce licenses for the operation of fish canneries and impose a fee for such licenses. The federal government, it appears, initiated the scheme as a necessary part of regulating the seacoast and inland fisheries. In short both the Supreme Court and the Privy Council struck down the federal licensing because the scheme was beyond the scope of federal jurisdiction.

The reference is a good example of the extent of the federal authority over the seacoast and inland fishery. The contention by the federal government that the licensing scheme was necessarily incidental to the protection and conservation of fisheries was rejected. It was pointed out that a cannery involves a trade process converting fish and fish products into marketable commodities (*2*, p. 119). Neither the Supreme Court nor the Privy Council could see how the protection and preservation of either the public right of fishing or proprietary fisheries had anything to do with civil rights of a person or persons to operate fish canneries. It was felt that actions that would relate to storage or possession of fish could be construed as being applicable to the seacoast and inland fisheries but not the business or operation of canning establishments. The Privy Council went on to point out that it could see no part of section 91 of the British North America Act 1867 giving the federal government the authority to license fish canneries (*2*, p. 199). Thus the attempt to license canneries was trenching on the provincial right to legislate on the subject of property and civil rights.

The important point raised here is the validity of federal legislation over fisheries. For federal schemes under the auspices of seacoast and inland fisheries to be held within federal scope they must be strictly related to fisheries as defined, or necessary to ensure the well-being of fisheries. Thus a tax on the tonnage of fish caught could be construed as a method to regulate for the protection of a fishery and would be allowed. However, it could be argued that if fees on fishing licenses were greater than necessary to recover the costs of administering the fees, then the purpose of the fees was not for the protection of fisheries but for economic gain. This being the case such a fee structure might be struck down as falling outside federal scope. The point that should be kept in mind is that for a federal government scheme to be upheld as valid under ection 91(12) of the BNA Act 1867, the seacoast and inland fishery, it must not appear to relate to anything that is not strictly relating to the regulation, protection, and preservation of fish and fisheries or to something that is not necessarily incidental to the regulation, protection, and preservation of fish and fisheries. Most of all federal government actions should not encroach upon a field or provincial authority unless it is clearly within federal authority to do so, or of "absolute" necessity.

Rex v Wagner and Rex v Tomasson et al. 1932

These two cases were appeals by the accused men, Wagner and Tomasson, against separate convictions for having in their possession fish that were out of season. The appeals were heard before the Manitoba Court of Appeal. While both men were accused of possession of a species of fish for which possession was illegal, there was an important distinction between the two cases. Tomasson had been convicted of being in contravention of a federal law outlawing

possession of the fish species in question, while Wagner had been convicted of violating a Manitoba statute prohibiting the same offense as did the federal law.

The majority of the Manitoba Court of Appeal found Tomasson of being in violation of the Fisheries Act (R.S.C. 1927, chapter 73) which had proclaimed a closed season on fishing and therefore would not allow his appeal against his prior conviction. The Court in doing so upheld the federal government's authority to regulate fisheries. The case illustrates an important aspect of the federal government's legislative jurisdiction over fisheries. The prohibition against fishing and possession of prohibited fish is in the realm of property and civil rights, a provincial matter, but even so the Court ruled the prohibition against possession was necessarily incidental to effective legislation on the matter of seacoast and inland fisheries. As such, the law was a proper enactment by the federal government. Thus it was acceptable for the federal government to limit a person's civil rights (the possession of fish) to ensure that an act, the regulation, protection, or preservation of a fishery, would be suitably effective.

Wagner on the other hand was convicted of being in violation of the Game and Fisheries Act of Manitoba (20–21 George V, chapter 15). On this occasion, the majority of the Justices of the Manitoba Court of Appeal allowed Wagner's appeal overturning his earlier conviction. The three Justices, in majority gave two reasons for their findings. C. J. M. Prendergast found that the Manitoba Legislation was "unqualifiedly" an act for the preservation of fish, and as such beyond the scope of provincial competence (*22*, p. 682–684). J. A. Dennistoun expressed another opinion, that the regulation of a closed season by the federal government left no room for a province to legislate on the same topic even from a different point of view, in this case the point of view of property (*22*, p. 687). Thus if the federal government legislates on a topic, the consequence is that any provincial legislation on the topic will have no force. The majority of the Court, J. A. Richards concurring with Prendergast and Dennistoun ruled that Wagner could not be convicted under the Manitoba Game and Fisheries Act. It appears that the Manitoba Court of Appeal at this time, did not favor the coexistence of similar federal and provincial legislation (*14*, p. 42).

The Wagner case does, however, point out an interesting proposition. In disagreeing with his three colleagues, J. A. Robson of the Manitoba Court of Appeal proposed that the province did have the right to legislate on closed seasons or on the manner of fishing. Robson's thesis appears to have been that he could not prohibit the granting or witholding their permission to fish, or assigning terms and conditions which a leasee or licensee should be required to follow persuant to the right to capture fish on crown property (*22*, p. 691). It appears that the thesis presented by Robson has some validity. Contemporary authorities likely favor the coexistence of federal and provincial legislation and would not readily accept the opinion expressed by the majority of the Court of Appeal (*14*, p. 42).

Clearly a province cannot enact *general* fisheries laws, for to do so would be trenching on the exclusive fisheries authority of the federal government. But the powers of the provinces cannot be construed so narrowly as to prohibit the provinces from legislating on matters that concern their property in fisheries. If the provincial legislation on the property or management appears similar, or the same as federal regulation, it can be argued that the provincial legislation is no less valid (*14*, p. 42), subject of course to the requirement that such legislation not be inconsistent to a federal enactment. Moreover, in this case it appears that the Privy Council's decision in the Fisheries Reference in 1898, makes provision for the provinces to have valid legislation on the property of fisheries. Their Lordships pointed out that it was competent for a province to declare a condition which a provincial fishery could be subject to when that fishery was to be "granted, leased, or otherwise disposed of" under the authority of section 92(5) and 92(13) of the BNA Act 1867, "The Management and Sale of Public Lands" and "Property and Civil Rights in the Province (*3*, p. 716).

In summary then, it appears that both federal and provincial governments can "regulate" the inland fisheries. There is a distinction though between the types of regulation. The federal government is regulating for the conservation and protection of fisheries, while the provinces through their right to legislate on property can regulate the terms and conditions that are to be respected in order to obtain and enjoy the privilege of fishing in provincial crown fisheries. It should be strongly emphasized, however, that provincial legislation must not be repugnant to federal legislation. Therefore, for example, a province could not require the use of a method of fishing that the federal government has disallowed.

Summary: Institutional Constraints

The Province of Manitoba was created in 1870, and the provinces of Saskatchewan and Alberta were created in 1905. However, it was not until 1930 that the federal government turned over the control of the natural resource to the three prairie

provinces. Before the Resource Transfer Agreements (BNA Act 1930, 20–21 George V, Chapter 26 (UK)), the federal government owned the Crown lands and natural resources of Manitoba, Saskatchewan, and Alberta as the Acts creating each of these provinces had excluded the ownership of land and resources from the domain of the provinces for reasons of 'National Policy (*20*, p. 24). Prior to 1930, therefore, the federal government exercised both proprietary rights and legislative jurisdiction over the fisheries which provided an absolute control over most aspects of the fisheries. A section of the B.N.A. Act 1930, however, changed the federal control of the prairie fisheries and put those provinces in the same legal standing with respect to the inland fisheries as Ontario and the other provinces. The Section read as follows:

"Except as herein otherwise provided, all rights of fishery shall, after the coming into force of this agreement, belong to and be administered by the province, and the Province shall have the right to dispose of all such rights of fishery by sale, license or otherwise, subject to the exercise by the Parliament of Canada of its legislative jurisdiction over sea-coast and inland fisheries." (BNA Act 1930, 20–21 George V, c. 26 (U.K.))

The purpose of this section, it appears, was to concede to Alberta, Saskatchewan, and Manitoba the rights in the inland fishery that Ontario had gained after 31 years of struggle. The federal government recognized that the inland fisheries were proprietary and that the provinces were entitled to exercise property rights and administrative functions in the fisheries. The federal government did, however, retain its exclusive and paramount legislative control over the fisheries of Canada whether seacoast or inland.

The result of this division is that there is a substantial overlap between the federal and provincial jurisdictions in the inland fisheries. While the federal government may regulate on fishing seasons, quotas, size limits, and tackle, the provincial governments have the right to determine who can fish, what privileges are conferred, and what fee they will pay to do so. Thus management tools in fisheries were divided between the two levels of government. The solution to this anachronism of our federal system of government has been achieved by delegating the responsibility for the administration of federal legislation to the provincial governments of the "Central Region." Thus, for example, the Fish and Wildlife Division of the Alberta Department of Lands and Forests administers both federal and provincial legislation with respect to fisheries. Furthermore, the provincial governments may recommend that changes be made to the federal fisheries regulation made under section 34 of the federal Fisheries Act (R.S.C. 1970, chapter F-14). Upon receipt of a request for amendment of legislation, the federal government will review the changes and examine the legalities before approval of amendments to fishery regulations. Approved amendments are passed by the Privy Council as Orders in Council and are gazetted to become law. At this time the provincial governments are responsible for the administration of the revised regulations pursuant to the federal Fisheries Act. Thus, the regulation of the inland fisheries is undertaken as a cooperative endeavor between the federal and provincial governments with the responsibility of administering the legislation delegated to the provinces. It would appear that any attempt to change this institutionalized procedure of management of fisheries, which has been in operation for a number of years, would undermine the cooperation that has existed.

As discussed in preceding sections, the federal government's authority under the seacoast and inland fishery is constrained by the terms of reference of the BNA Act 1867 and the subsequent legal interpretations of the Act in the courts. However, it is interesting to note that the federal government exercises indirect control over fisheries under its authority to provide for all matters pertaining to trade and commerce (BNA Act 1867, 30–31 Victoria, chapter 3 (UK), section 91(2)). Here again an overlapping of jurisdiction arises between the two levels of government. The provincial governments may regulate trade and commerce within provincial boundaries as a matter of property and civil rights (Ibid., section 92(13)). The distinction between the two levels of jurisdiction is that products that are intended for interprovincial or export trade are subject to federal legislation while products that are to be traded primarily within a province are subject to the province's regulation (*5*, p. 5).

Again the federal and provincial governments have chosen to use complementary legislation to avert the problems that can arise where federal–provincial authority overlaps. For example, the marketing of fish in most of the "Central Region" is handled by the Freshwater Fish Marketing Act (R.S.C. 1970, chapter F-13), an Act of the Parliament of Canada to regulate interprovincial and export marketing of freshwater fish. The Province of Manitoba in its Fisheries Act of 1969 (2nd session, chapter 9) uses its authority to make regulations for fish marketing in Manitoba by stating that federal regulations will apply in the province as if they were enacted by the provincial legislature. This type of agreement also has been made by Newfoundland and Nova Scotia with regards to the Saltfish Act of 1970 (R.S.C. 1970, chapter 37, 1st suppl.).

While it appears more or less clear that the management prerogatives under the present system of federal–provincial cooperation have been delegated to the provincial government, the federal government maintains a constitutional responsibility to provide for the regulation, protection, and preservation of all fisheries in Canada. As Mr Justice Fournier said in 1882 it is the federal government's responsibility to keep fisheries from ruin and decay and to police fisheries such that they are developed and conserved for the general profit of Canada (*2*, p. 141). Thus while the provinces exercise proprietary rights and have been delegated responsibility for the administration of federal legislation it remains the federal government's responsibility to ensure that the fisheries are protected and that they profit Canadians. Given the institutional constraint to resource management it is apparent that a number of serious problems may arise from federal attempts to institute economic considerations into fisheries management, for instance obtaining a rent from the fisheries resource. In the past, federal attempts to collect fees from federal licensing systems have been struck down by the courts. Resource royalties from the inland fisheries generally accrue to the provinces. Although the federal government has almost unlimited taxing authority, and while it may be within the power of the federal government to design a system of taxation to capture a financial return from the inland fisheries, such an attempt would most certainly provoke the ire of the provinces. The proprietorship over natural resources in Canada has traditionally included the right of the provincial crowns to appropriate the revenues that arise from the natural resources. Thus federal taxation schemes, such as the federal export tax on Alberta oil, which capture revenues that would otherwise go into provincial treasuries, will more than often receive severe criticism from the provinces. Moreover, the provinces, as the owners of the resources, by exercising their proprietary rights, will often have the ability to thwart many federal economic undertakings if they are inclined to do so. Provincial reactions of this kind can only be detrimental to all the parties concerned in resource management. Therefore the management of the provincial inland fisheries must be on a federal–provincial cooperative level.

It is apparent that the fisheries resource management activity of the federal government within the provincial boundaries of the "Central Region" will generally be of an extension type service. The activity of the Inspection Branch of the Fisheries and Marine Service represents an exception to this observation. The cooperative arrangement of management and the legal framework of the inland provincial fisheries demand that this be so. However, the federal government, by virtue of its complete management control over the fisheries of the Northwest Territories is directly involved with resource allocation decisions and the economic implications of such decisions.

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The Structure of Marine Ecosystems. *John H. Steele. Harvard University Press, Cambridge, Mass. 1974. 128 p., $7.95.*

In this very elegant little book John Steele has used a simulation model of events in a marine planktonic food web as the focus of his writing. He has dealt with many questions currently of interest to ecologists, such as: what is the influence of species diversity or environmental diversity on the stability of ecosystems? What is the importance of trophic relationships and how should they be handled in models? Which human impact is more important in the sea, overfishing or pollution?

The book falls roughly into three parts: a discussion of basic ecological principles as they apply to marine planktonic systems, an account of the construction and operation of a simulation model using data from the North Sea, and a chapter of "Speculations" in which he uses insights derived from the model, together with his extensive knowledge of marine ecology, to illuminate problems of practical interest. Throughout the book he stresses the comparison of terrestrial with marine systems.

Some solutions are offered to problems which currently vex the practicing ecologist. For example, how does one fit animals which feed at several trophic levels into a model which requires each animal to be assigned to one trophic level? John Steele assigns the species or group to the lowest of its possible trophic levels and regards other feeding practices as subsidiary loops of the food web. To the question "What is the relation between diversity and stability in a system?", the author replies with appropriate qualifications, that neither diversity nor efficiency has any relation to the dynamic stability of a system.

The model is used in a very intelligent way, with due recognition of its limitations. While he succeeds in producing a rough simulation of conditions in the North Sea, he shows clearly where the model rests on weak assumptions and where alternative constructions could have been used, with differing results. One of his most important conclusions is that certain types of behavioral response, such as ceasing to feed when the concentration of food falls below a threshold level, can have an invaluable stabilizing effect on marine systems. He stresses that the model is much simpler than the natural situation, and that his conclusion only draws attention to the need for further experiments on threshold responses.

In comparing marine and terrestrial systems, the key observation is that on land usually less than 10% of plant production is grazed by herbivores, while in the plankton this proportion is much higher. Hence, it follows that terrestrial herbivores are seldom food-limited in a density dependent manner, while in the sea they often are. He is careful to point out that the macrophytes of the near-shore environment are "terrestrial" in this respect, while arctic lemming cycles are almost "marine." He also makes the point that the efficiency of terrestrial systems in converting plant production into animal food for man appears to be much lower than the efficiency of marine systems such as the North Sea.

The final section suggests numerous fruitful lines of enquiry: for example, it highlights the inadequacy of our knowledge of processes in seabed communities and compares the effects of overfishing with the effects of various forms of pollution.

In general there is no doubt that, used in this way, simulation models of ecosystems are an excellent technique for integrating diverse information and highlighting areas in need of experimental study. This is a refreshing change from the uncritical use of simulation models for purposes of prediction, and ecology will benefit greatly from further studies of this type.

KENNETH H. MANN

Department of Biology
Dalhousie University
Halifax, N.S.

(R19)

CORRECTIONS TO RECENT PUBLICATIONS

CORRECTIONS SUR RÉCENTES PUBLICATIONS

VOLUME 31, No. 9 — paper by J. E. Paloheimo and P. F. Elson: *Reduction of Atlantic salmon (Salmo salar) catches in Canada attributed to the Greenland fishery.*

Page 1473: Right column, line 2, "16–24%" should read "13–24%"; line 3, "20–52%" should read "15–52%."

BULLETIN 184 — by W. B. Scott and E. J. Crossman: *Freshwater Fishes of Canada*, 1973, 966 p.

An errata sheet is available upon request from the Office of the Editor.

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Identification of *Anisakis* larva (I) as *Anisakis simplex* (Rudolphi, 1809, det. Krabbe 1878) (Nematoda:Ascaridata) **J. H. C. Pippy and P. van Banning**

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Effects of thiourea treatment on sexually maturing and gonadectomized male sockeye salmon (*Oncorhynchus nerka*) **J. R. McBride and A. P. van Overbeeke**

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Notes

Infectious pancreatic necrosis virus and bacterial kidney disease appearing concurrently in populations of *Salmo gairdneri* and *Salvelinus fontinalis* **T. Yamamoto**

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Notes: Differ from papers on the basis of scope or completeness, rather than quality of research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on methodology or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as articles except that they have no centered headings and should occupy no more than four printed pages.

Reviews: Detailed articles that are critical appraisals of broad areas of investigation, particularly in fields that have shown rapid development in recent years.

Perspectives: Essays of opinion or hypothesis on fisheries, ocean science, aquatic environments, or related topics that are of concern to the professional and layman. References are desirable; informal style is welcomed.

Letters: Opinion on topics recently under discussion in the *Journal*; preferred maximum length, 500 words. The Editor reserves the right to shorten letters and to make other changes to accord with the style of the *Journal*.

Editorial Practices

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW., Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopedique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970) Special Publication No. 6 of the American Fisheries Society.

Series titles should be avoided. An *abstract* is required for all manuscripts, and should state concisely in less than 250 words, what was done, the essentials of the new knowledge, and the main conclusions. Abstracts are published in English and French; those submitted in one language only are translated by the *Journal*.

The Editor normally submits each manuscript to at least two *referees*. Contributors are encouraged to suggest at least four names to be added to the list from which referees may be selected.

Illustrations should be submitted in triplicate as photographs (*glossy prints*) no larger than a standard page and must accompany the manuscript. Prints should not be mounted, stapled, or clipped. Symbols, letters and numerals must be neat, and at least 1.5 mm high when the illustration is reduced to the appropriate size. Drawings should, when possible, be grouped to facilitate comparisons.

Proofs are sent to the author, who is responsible for correcting only errors in typesetting. *Alterations to the content must be avoided.*

Fifty *reprints*, without covers, are supplied free. Additional copies may be ordered when the galley proof is returned. A charge of $30.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the biology?

Would any of the text be clearer if condensed? Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

Journal Fisheries Research Board Canada

Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

Sujets

Nous désirons recevoir des manuscrits s'insérant dans le vaste domaine de la gestion des pêches, des sciences de la mer et des milieux aquatiques au Canada.

***Articles*:** Comptes rendus d'études d'une portée générale, constituant des contributions inédites à la science.

***Notes*:** Se distinguent des articles par la portée et le degré d'achèvement plutôt que par la qualité des recherches. Elles peuvent traiter de problèmes particuliers et bien définis; de travaux qui sont en grande partie confirmatoires; de méthodologie ou de techniques de recherche; de progrès de la connaissance découlant d'études plus vastes; de développements dans des projets abandonnés; ou de découvertes préliminaires qui méritent d'être publiées avant la solution de problèmes à l'étude. Les notes peuvent être présentées d'une façon aussi souple que les articles, mais elles ne doivent pas porter d'en-têtes centrés ni occuper plus de quatre pages.

***Critiques*:** Articles détaillés constituant une appréciation critique de vastes champs de recherches, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

***Perspectives*:** Essais de l'opinion ou de l'hypothèses sur les pêches, les sciences de la mer, les milieux aquatiques, ou sur des sujets connexes qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans un style simple.

***Lettres*:** Opinions sur des sujets exposés dans le *Journal*. Longueur maximale préférable: 500 mots. Le directeur se réserve le droit de raccourcir les lettres et de faire d'autres modifications dans le style du *Journal*.

Dispositions générales

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Il faut éviter les *articles en série*. Un *résumé* doit accompagner chaque texte et doit exposer brièvement (moins de 250 mots) le travail accompli, les points essentiels des connaissances acquises et les principales conclusions. Les résumés paraissent en anglais et en français, le *Journal* veillant à la traduction dans le cas des présentations unilingues.

Le directeur soumet d'ordinaire chaque texte à l'approbation de deux *arbitres* ou plus. Nous encourageons les collaborateurs à suggérer au moins quatre personnes dont les noms pourraient figurer sur la liste des arbitres.

Les *illustrations* doivent être sous forme de photos (*sur papier glacé*), ne pas dépasser les dimensions d'une page normale et accompagner le texte. Elles ne doivent pas être montées, coupées ni brochées. Les symboles, lettres ou chiffres doivent se détacher avec précision et avoir au moins 1,5 mm de hauteur lorsque l'illustration est réduite à la grandeur voulue. Les dessins doivent, autant que possible, être groupés pour faciliter l'étude comparative.

L'auteur doit revoir les *épreuves* qu'on lui remet et en corriger seulement les erreurs typographiques. *Il doit éviter de changer le contenu du texte.*

Cinquante *tirés à part*, sans couverture, sont fournis gratuitement. L'auteur peut profiter du renvoi des épreuves en placards pour commander des tirés à part supplémentaires. Les frais sont de $30.00 par page pour la publication des articles. Quand un auteur soumet un manuscrit, il doit spécifier que son institution accepte les frais.

Listes de contrôles pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

Journal Fisheries Research Board Canada

Guide for Typists Preparing Copy

Read "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and two other clear copies to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C).
Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send at least 3 *glossy prints* (no larger than 21.5 × 28 cm) of each illustration with the manuscript. Type the author's name and figure number on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the numeral *one*, and between the capital letter $\underline{O}$ and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

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Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21.5 × 28 cm (8.5 × 11 pouces), laissant toujours une marge d'au moins 2.5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les minuscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation de ou des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et ainsi que deux copies lisibles au bureau du Directeur de la publication.

Tableaux. Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).

Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par les minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Envoyez avec le manuscrit trois photos *sur papier glacé* (21,5 × 28 cm ou moins) pour chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le Journal (voir Appendice E). Les noms de revues sont abrégés suivant les *Biological Abstracts*; en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

8th International Conference of the International Association on Water Pollution Research

Sydney, Australia, August 22–27, 1976

The Scientific Program of the 8th Conference is expected to provide for two streams of concurrent plenary sessions on each of eight half days during the week. Simultaneous interpretation will be available for these plenary sessions as well as for the Opening and Closing Ceremonies.

Those authors who wish to submit papers for consideration by the 8th Conference Selection Committee, are advised that their subject matter should fall within the general field of water pollution research but with particular emphasis on: 1, discharge to coastal and estuarine waters, 2, land management and water quality objectives, 3, treatment of food and agricultural wastes in rural areas, 4, identification and documentation of water pollution situations, 5, relevant measures for effective pollution control. Papers will be selected on the basis of originality, status of the work described, the importance of the subject and its relevance to the areas outlined above. Seven copies of each paper should be supplied. A high standard of presentation will be expected in the papers, which will be refereed by five experts. Only completed papers can be considered.

For further information contact:

I.A.W.P.R. 8th International Conference
Office of the Secretariat
G.P.O. Box 2609
Sydney N.S.W. 2001, Australia
Cable to conventions Sydney
telephone (code 02) 27-6940

8e Conférence internationale de l'Association internationale sur la recherche en pollution de l'eau

Sydney (Australie), du 22 au 27 août 1976

On s'attend à ce que le programme scientifique de la 8e Conférence comprenne deux séries d'assemblées plénières parallèles qui se tiendront les huit demi-journées de la semaine. Un service d'interprétation simultanée sera offert à ces assemblées ainsi qu'à l'ouverture et à la fermeture des cérémonies.

Les auteurs intéressés à soumettre des rapports, qui seront étudiés par le Comité de sélection de la 8e Conférence, doivent choisir un sujet qui porte sur le domaine général de la recherche en pollution de l'eau et mettre l'accent sur: 1. le déversement dans les eaux côtières et d'estuaires, 2. les objectifs de la gestion des terres et de la qualité de l'eau, 3. le traitement des déchets alimentaires et agricoles dans les secteurs ruraux, 4. l'identification et la documentation relatives à la situation de la pollution de l'eau et 5. les mesures propres à la dépollution efficace. Pour choisir les documents, on se basera sur l'originalité, l'état du travail décrit, l'importance du sujet et sa pertinence aux secteurs décrits ci-dessus. Il faut fournir sept copies de chaque document. La présentation est très importante et sera jugée par cinq experts. On ne tiendra compte que des documents complétés

Pour de plus amples renseignements, prière de communiquer avec:

I.A.W.P.R. 8th International Conference
Office of the Secretariat
G.P.O. Box 2609
Sydney N.S.W. 2001, Australia
Cable to conventions Sydney
téléphone (code 02) 27–6940

Second International Symposium on Environmental Biogeochemistry
April 8-11, 1975

Canada Centre for Inland Waters Burlington, Ontario, Canada

OBJECTIVES: The purpose of the Symposium will be to review some aspects of biogeochemical processes as they pertain to the human environment. The theme of the meeting will be the effects of man on the carbon cycle and the other key elemental cycles.

PROGRAM: Topics will include (but not be restricted to) biogeochemical processes in waters at all levels of salinities, the role or organic materials, and the influence of atmospheric processes on the ecology of soils, sediments, and waters.

Selected papers will be arranged in the following technical sessions:

I — Ecosystem studies of element, energy and mass balances.
II — Effects of man on the Carbon Cycle.
III — Dynamics and biotransformations of phosphorus, sulfur, and nitrogen in ecosystems.
IV — Transformation of metals within ecosystems.

REGISTRATION FEE: $35 (preregistered by March 1, 1975)
$40 (if registered at meeting)

Abstract and requests for information should be addressed to:

Dr. Jerome O. Nriagu
Department of the Environment
Canada Centre for Inland Waters
Burlington, Ontario L7R 4A6
Canada
Tel.: (416) 637–4252

Second colloque international sur la biogéochimie de l'environnement
du 8 au 11 avril 1975

Centre canadien des eaux intérieures Burlington (Ontario) Canada

OBJECTIFS: Le colloque a pour objet d'examiner certains aspects des processus biogéochimiques reliés à l'environnement humain. Le thème de la réunion sera l'influence de l'homme sur le cycle du carbone et sur les autres cycles d'éléments très importants.

PROGRAMME: Les sujets porteront (entre autres) sur les processus biogéochimiques dans les eaux de tous les niveaux de salinité, le rôle des matières organiques et l'influence des processus atmosphériques sur l'écologie des sols, des sédiments et des eaux.

Les études choisies seront présentées au cours des sessions techniques suivantes:

I — études des bilans des éléments, de l'énergie et de la matière dans l'écosystème;
II — action de l'homme sur le cycle du carbone;
III — dynamique et réactions biochimiques du phosphore, du soufre et de l'azote dans les écosystèmes; et
IV — réactions chimiques des métaux dans les écosystèmes.

FRAIS D'INSCRIPTION: $35 (avant le 1er mars 1975)
$40 (à la réunion)

Les résumés et les demandes de renseignements doivent parvenir à:

Monsieur Jérôme O. Nriagu
Ministère de l'Environnement
Centre canadien des eaux intérieures
Burlington (Ontario) L7R 4A6
Canada
Tel.: (416) 637–4252

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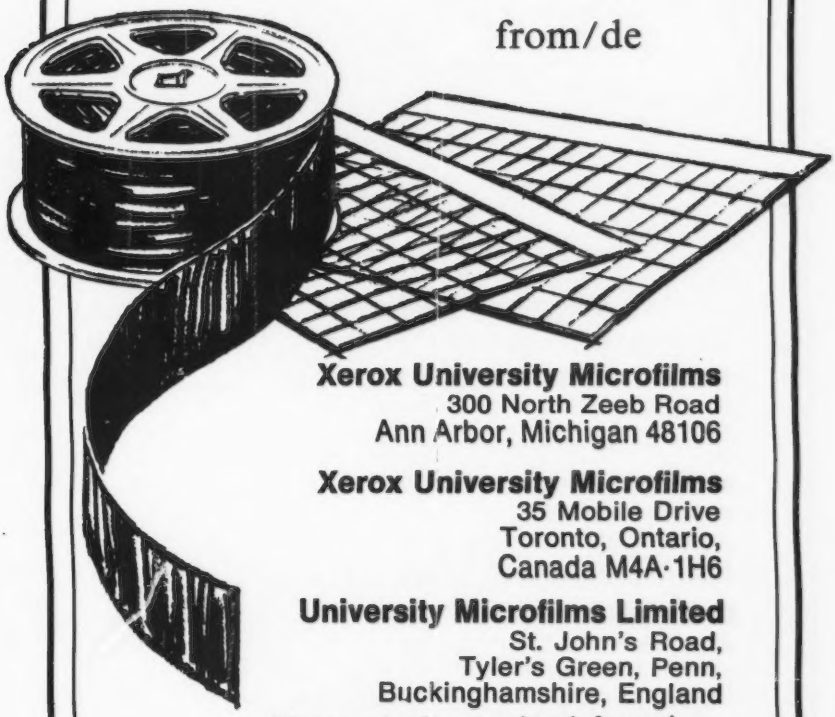

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Journal of the Fisheries Research Board of Canada

Volume 31, No. 12, December 1974 JFRBAK31(12)1849–1999(1974)

Volume 31, N° 12, décembre 1974

Announcement/Avis J. C. Stevenson 1849

Identification, analysis, and removal of geosmin from muddy-flavored trout M. Yurkowski and J. Tabachek 1851–1858

Theory of size distribution in ecological communities S. R. Kerr 1859–1862

Redescription of *Anonchohaptor anomalum* Mueller 1938 and a description of *Icelanonchohaptor fyviei* n.sp. (Monogenoidea: Dactylogyridae) A. O. Dechtiar and W. A. Dillon 1863–1866

Behavior of juvenile coho salmon (*Oncorhynchus kisutch*) exposed to Sumithion (fenitrothion), an organophosphate insecticide C. J. Bull and J. E. McInerney 1867–1872

Effectiveness of vancomycin against gaffkemia, the bacterial disease of lobsters (genus *Homarus*) J. E. Stewart and B. Arie 1873–1879

Photochemical dechlorination of water supply for fish tanks with commercial water sterilizers F. A. J. Armstrong and D. P. Scott 1881–1885

Comparison of various vaccines for inducing resistance in the lobster *Homarus americanus* to the bacterial infection, gaffkemia J. E. Stewart and B. M. Zwicker 1887–1892

Biochemical, physiological, and pathological changes in pyridoxine-deficient rainbow trout (*Salmo gairdneri*) C. W. Smith, M. Brin, and J. E. Halver 1893–1898

Early development and distribution of the flathead sole (*Hippoglossoides elassodon*) D. F. Alderdice and C. R. Forrester 1899–1918

Observations on subsurface distributions of chlorophyll *a* and phytoplankton carbon in the northeast Pacific Ocean L. A. Hobson and D. E. Ketcham 1919–1925

Notes

Three-dimensional computer graphics in fisheries science D. J. Brown and L. L. Low 1927–1935

Retarded behavioral development and impaired balance in Atlantic salmon alevins (*Salmo salar*) hatched from gastrulae exposed to DDT P. A. Dill and R. C. Saunders 1936–1938

Serological diagnosis of corynebacterial kidney disease of salmonids P. K. Chen, G. L. Bullock, H. M. Stuckey, and A. C. Bullock 1939–1940

Social conditions increase intermolt period in juvenile lobsters *Homarus americanus* J. S. Cobb and G. R. Tamm 1941–1943

A hermaphroditic lake whitefish, *Coregonus clupeaformis*, from Lake Huron T. R. Porter and S. Corey 1944–1945

Hunger and the capture of grayling and char J. E. Bryan 1945–1948

Toxicity of copper at two temperatures and three salinities to the American lobster (*Homarus americanus*) D. W. McLeese 1949–1952

Changes in blood chemistry of juvenile steelhead trout, *Salmo gairdneri*, following sublethal exposure to nitrogen supersaturation T. W. Newcomb 1953–1957

Indirect fluorescent antibody technique for detection of RM bacterium of rainbow trout (*Salmo gairdneri*) G. R. Johnson, G. Wobeser, and B. T. Rouse 1957–1959

DDT and the rugophilic response of settling barnacles *Balanus improvisus* N. Meith-Avčin 1960–1963

Perspectives

Institutional constraints in fisheries management P. C. Thompson 1965–1981

Book review/Compte rendu 1983

Corrections to recent publications/Corrections sur récentes publications 1984

Coming next month/A paraître le mois prochain 1985

Recent publications/Publications récentes 1987–1998

Additional publications/Publications additionnelles 1999

Guide to Authors and Typists/Guide des auteurs et Guide pour les secrétaires d'auteurs *i-v*

INFORMATION CANADA, OTTAWA, 1974